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(54) Title: METHODS AND COMPOSITIONS FOR THE TREATMENT OF CELL PROLIFERATION

(57) Abstract: The present invention relates to a use of a composition comprising one or more inhibitors of the citric acid, TCA, cycle and/or one or more inhibitors of glycolysis for the manufacture of a medicament for the treatment of cellular proliferation, wherein said composition is administered into a mass of the proliferating cells.

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METHODS AND COMPOSITIONS FOR THE TREATMENT OF CELL PROLIFERATION

BACKGROUND TO THE INVENTION

5 The treatment of conditions relating to cellular proliferation, malignant and benign, such as tumours, hyperproliferative scars, cheloid scars, and restenotic processes at the level of a duct have several disadvantages, such as, for example, high toxicity, low efficacy, expense and the requirement for repeated or continuous administration.

10 The use of metabolic pathway inhibitors for the treatment cellular proliferation is known in the prior art. For example, US 2003/30181393 describes inhibitors of glycolysis and oxidative phosphorylation; US 2003/0087961 described the use of inhibitors of glycolysis; EP 1372646, WO 02/072077, WO 2004/024676 described the use of glycolysis and transaminase inhibitors; US2002/0187534 and US2002/0024050 describe the blocking of fatty acid synthase to inhibit cellular proliferation.

15 The present invention aims to overcome the problems of the prior art by providing alternative an improved treatments for cellular proliferation.

SUMMARY OF SOME EMBODIMENTS OF THE INVENTION

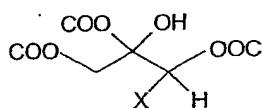
20 One embodiment of the present invention is a use of a composition comprising one or more inhibitors of the citric acid, TCA, cycle and/or one or more inhibitors of glycolysis for the manufacture of a medicament for the treatment of cellular proliferation, wherein said composition is administered into a mass of the proliferating cells.

25 One embodiment of the present invention is a use of a composition as described above, wherein said TCA cycle and glycolysis inhibitors are administered separately, simultaneously or sequentially .

30 Another embodiment of the present invention is a use of a composition as described above, wherein said TCA cycle inhibitor is an inhibitor of one or more of pyruvate dehydrogenase, citrate synthase, aconitase, isocitrate lyase, alpha-ketoglutarate dehydrogenase complex, succinyl CoA synthetase, succinate dehydrogenase, fumarase, malate synthase, glutaminase and pyruvate dehydrogenase complex.

Another embodiment of the present invention is a use of a composition as described above, wherein said TCA cycle inhibitor is any of arsenite, hypoglycin A, methylenecyclopropylacetic acid, alloxan, PNU, p-benzoquinone, fluoroacetate, halogenated acetates (iodo-, bromo-, chloro-acetate), halogenated acetyl-CoA (fluoroacetyl-CoA, bromoacetyl-CoA, chloroacetyl-CoA, iodoacetyl-CoA), halogenated crotonate (fluoro-, iodo-, bromo-, chloro-crotonate), halogenated ketone bodies, (chloro-, fluoro-, bromo-, iodoaceto-acetate, fluoro-, chloro-, bromo-, iodo-butyrate, fluoro-, chloro-, bromo-, iodo-acetone), halogenated oleate (iodo-, bromo-, chloro-, fluoro-oleate), halogenated citrate, halogenated citrate 2R, 3R isomer (fluoro-, bromo-, chloro-, iodo-citrate), dichlorovinyl-cysteine, halogenated aminoacids, malonate, pentachlorobutadienyl-cysteine, 2-bromohydroquinone, 3-nitropropionic acid, cis-crotonalide fungicides, glu-hydroxyoxamate, p-chloromercuriphenylsulphonic acid, L-glutamate gamma-hydroxamate, p-chloromercuriphenylsulphonic acid, acivicin (alpha-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid), halogenated glutamine (fluoro, iodo, chloro, bromo-glutamine), or halogenated glutamate (fluoro, iodo, chloro, bromo-glutamate), a stereoisomer, tautomer, racemate, prodrugs, metabolite thereof, or a pharmaceutically acceptable salt, base, ester or solvate thereof.

Another embodiment of the present invention is a use of a composition as described above, wherein said TCA cycle inhibitor is a compound of formula (I) or a stereoisomer, tautomer, racemate, prodrug, metabolite thereof, or a pharmaceutically acceptable salt, base, ester or solvate thereof:



(I)

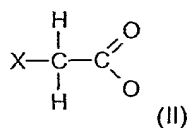
where X is halide, a sulfonate, a carboxylate, an alkoxide, an amine oxide or OH.

Another embodiment of the present invention is a use of a composition as described above, wherein formula (I):

- a halide is selected from the group consisting of: fluoride, bromide, chloride, and iodide,
- a sulfonate may be selected from the group consisting of: triflate, mesylate and tosylate,

- a carboxylate may be selected from the group consisting of: methoxylate and ethoxylate,
- an alkoxide may be selected from the group consisting of: methoxide and ethoxide,
- an amine oxide is dimethylamine oxide, and
- 5 - where the stereochemistry is 2R, 3R,

Another embodiment of the present invention is a use of a composition as described above, wherein said TCA cycle inhibitor is a compound of formula (II) or a stereoisomer, tautomer, racemate, prodrug, metabolite thereof, or a pharmaceutically acceptable salt, base, ester or
 10 solvate thereof:



where X is a halide, a sulfonate, a carboxylate, an alkoxide, an amine oxide, or an OH.

- 15 Another embodiment of the present invention is a use of a composition as described above, wherein formula (II):
- the halide is selected from the group consisting of: fluoride, bromide, chloride, and iodide,
 - the sulfonate is selected from the group consisting of: triflate, mesylate and
 20 tosylate,
 - the carboxylate is selected from the group consisting of: methoxylate and ethoxylate,
 - the alkoxide is selected from the group consisting of: methoxide and ethoxide, and
 - the amine oxide is dimethylamine oxide.

25

9 Another embodiment of the present invention is a use of a composition as described above, wherein an inhibitor of the TCA cycle is any of fluoroacetate, fluorocitrate, arsenite, acetoacetate, and betahydroxy butyrate.

- 30 Another embodiment of the present invention is a use of a composition as described above, wherein said TCA cycle inhibitor, when comprising a halogen atom, comprises a halogen radioisotope in place of a stable halogen atom.

Another embodiment of the present invention is a use of a composition as described above, wherein said inhibitor of glycolysis inhibits at least one enzyme from the group consisting of hexokinase, glucokinase, phosphoglucose isomerase, phosphofructokinase, aldolase, triose phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglyceromutase, enolase, pyruvate kinase, and lactate dehydrogenase.

Another embodiment of the present invention is a use of a composition as described above, wherein said inhibitor of glycolysis is a hexose sugar modified by removal of the hydroxyl group or by the substitution of the hydroxyl group with halogen atom or thiol at:

- C6,
- C1 or C2 or C5,
- C3 and/or C4, and/or
- C2 or C3.

Another embodiment of the present invention is a use of a composition as described above, wherein said inhibitor of glycolysis is any of 6-deoxy-6-fluoro-D-glucose, 6-deoxy-6-bromo-D-glucose, 6-deoxy-6-chloro-D-glucose, 6-O-methyl-D-glucose, 6-thio-D-glucose, 6-deoxy-D-glucose, C-6 modified or blocked derivatives of other hexose ring pyranoses, mannopyranoses, galactopyranoses, 6-deoxy-6-fluoro-D-glucose, 6-deoxy-6-bromo-D-mannose, 6-deoxy-6-chloro-D-mannose, 6-deoxy-6-fluoro-D-galactose, 6-deoxy-6-chloro-D-galactose, 6-deoxy-6-iodo-D-galactose, 6-deoxy-6-bromo-D-galactose, halogenated C-6 sugars gluconolactones, glucuronic acid, glucopyranoside, and their phosphate derivatives, glucuronides with halogenated glycosides at the C-1 position, C-2 substituted D-hexoses, 2-deoxy-2-halogeno-D-hexoses, 2-deoxy-2-fluoro-D-glucose, 2-chloro-2-deoxy-D-glucose, 2-bromo-D-glucose, 2-iodo-D-glucose, 2-deoxy-2,2-difluoro-D-arabino-hexose, 2-deoxy-2-fluoro-D-mannose, 2-deoxy-D-arabino-hexose, 2-Deoxy-2-fluoro-D-galactose, 1,6-anhydro-2-deoxy-2-fluoro-beta-D-glucopyranose, 1-6-anhydrosugar, 2-amino-2-deoxy-D-glucose, glucose amine, 2-amino-2-deoxy D-galactose, galactosamine, 2-amino-2-deoxy-D-mannose, mannosamine, 2-deoxy-2-fluoro-D-mannose, 2-deoxy-2-fluoro-D-galactose, 2-deoxy-D-arabino-hexose, 2-deoxy-2,2-difluoro-D-arabino-hexose, 2-deoxy-2-fluoro-D-glucose 1-Phosphate, 2-deoxy-2-fluoro-D-glucose 6-P, 2-deoxy-2-fluoro-D-glucose 1,6 biphosphate, 2-deoxy-2-fluoro-D-mannose 1-P, 2-deoxy-2-fluoro-D-mannose 6-P, 2-deoxy-2-fluoro-D-mannose 1,6-biphosphate, nucleotide diphosphate, uridine di-P, 1-2 deoxy-2-fluoro-D-glucose, C-2-halogen substituted, and NH₃ substituted derivatives of D-Glucose 6-

phosphate, 2-deoxy-2-fluoro-2-D-glucose-6-phosphate, 2-chloro-2-deoxy-D-glucose-6-phosphate, 2-deoxy-D-arabino-hexose-6-phosphate, D-glucosamine-6-phosphate, 2-deoxy-2-fluoro-2-D-mannose-6-P, and any known derivatives, C-2 halogenated derivatives of hexose ring pyranoses, mannopyranoses, galactopyranoses, C-2-deoxy-2-fluoropyranoses, and any derivative, C-2 halogenated sugars derivatives, C-2 fluoro-, bromo-, chloro-, or iodo-sugars derivatives, fluoro, bromo, chloro, or iodo C-2 sugars derivatives, gluconolactones, glucuronic acid, glucopyranoside, and their phosphate derivatives, sugars modified at C-1 or C-5 by replacement of hydroxyl by fluorine or deoxygenation or replacement by a sulfur group, glucosyl fluoride, 1-deoxy-D-glucose, 5-thio-D-glucose, 3-deoxy or 3-fluoro-D-glucose or 4-deoxy or 4-fluoro-D-glucose, 2-fluoro- or 2-iodo-, or 2-thio-, or 2-methoxy- or 3-fluoro-, or 3, 3 difluoro-, 3-iodo-, or 3-carboxylo-, or 3-thio-glyceraldehydes or glycerates, 3-fluoro-2-phosphoglycerate, phosphothioesters or other phosphor modified analogs, mannoheptulose mannoheptose, glucoheptose, N-acetylglucosamine, 6-aminonicotinamide acidosis-inducing agents, 2-deoxy-2-fluoro-D-glucose, citrate and halogenated derivatives of citrate, fructose 2,6-bisphosphate, bromoacetyethanolamine phosphate analogues, N-(2-methoxyethyl)-bromoacetamide, N-(2-ethoxyethyl)-bromoacetamide, N-(3-methoxypropyl)-bromoacetamide), iodoacetate, pentalenolactone, arsenic, 1,1-difluoro-3-phosphate-glycerol, oxamate, 2-fluoro-propionic acid or it salts, 2,2-difluoro-propionic acid, 3-halopropionic acid, or 2-thiomethylacetic acid, a stereoisomer, tautomer, racemate, prodrug, metabolite thereof, or a pharmaceutically acceptable salt, base, ester or solvate thereof.

Another embodiment of the present invention is a use of a composition as described above, wherein an inhibitor of glycolysis is any of 2FDG, oxamate or iodoacetate or a stereoisomer, tautomer, racemate, prodrug, metabolite thereof, or a pharmaceutically acceptable salt, base, ester or solvate thereof.

Another embodiment of the present invention is a use of a composition as described above, wherein said glycolysis inhibitor, when comprising a halogen atom, comprises a halogen radioisotope in place of a stable halogen atom.

Another embodiment of the present invention is a use of a composition as described above, wherein said composition further comprises pyrophosphate.

Another embodiment of the present invention is a use of a composition as described above, wherein said pyrophosphate is one or more of sodium pyrophosphate, potassium pyrophosphate, calcium pyrophosphate.

- 5 Another embodiment of the present invention is a use of a composition as described above, wherein said pyrophosphate is administered simultaneous, separate or sequentially in respect of the other components of the composition.

- Another embodiment of the present invention is a use of a composition as described above,
10 wherein said composition further comprises one or more imaging agents

Another embodiment of the present invention is a use of a composition as described above, wherein said imaging agent is any of poly(ortho)ester, metallic powder, magnesium alloy powder, tantalum powder, biocompatible metal powder, iridium powder, or micro-bubbles.

- 15 Another embodiment of the present invention is a use of a composition as described above, wherein said composition further comprises one or more slow releasing agents.

- Another embodiment of the present invention is a use of a composition as described above,
20 wherein said slow release agents is a polymer that is any of magnesium alloy, poly(glycolic acid, poly(lactic acid) or in general glycolic- and lactic acid based polymers, copolymers, poly caprolactones and in general, poly hydroxyl alkanoate,s poly(hydroxy alcanoic acids), Poly (ethylene glycol), poly vinyl alcohol, poly (orthoesters), poly (anhydrides), poly (carbonates), poly amides, poly imides, poly imines, poly (imino carbonates), poly (ethylene imines),
25 polydioxanes, poly oxyethylene (poly ethylene oxide), poly (phosphazenes), poly sulphones, lipids, poly acrylic acids, poly methylmethacrylate, poly acryl amides, poly acrylo nitriles (Poly cyano acrylates), poly HEMA, poly urethanes, poly olefins, poly styrene, poly terephthalates, poly ethylenes, poly propylenes, poly ether ketones, poly vinylchlorides, poly fluorides, silicones, poly silicates (bioactive glass), siloxanes (Poly dimethyl siloxanes),
30 hydroxyapatites, lactide-capronolactone, poly aminoacids (natural and non natural), poly β -aminoesters, albumines, alginates, cellulose / cellulose acetates, chitin / chitosan, collagene, fibrine / fibrinogen, gelatine, lignine, proteine based polymers, Poly (lysine), poly (glutamate), poly (malonates), poly (hyaluronic acids), Poly nucleic acids, poly saccharides, poly (hydroxyalkanoates), poly isoprenoids, starch based polymers, copolymers thereof,
35 linear, branched, hyperbranched, dendrimers, crosslinked, functionalised derivatives thereof,

hydrogels based on activated polyethyleneglycols combined with alkaline hydrolyzed animal or vegetal proteins.

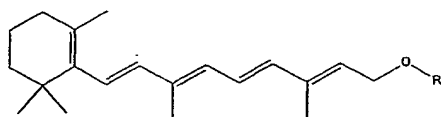
Another embodiment of the present invention is a use of a composition as described above,
 5 wherein at least one of said inhibitors is coupled to solubilising agent.

Another embodiment of the present invention is a use of a composition as described above,
 wherein said solubilising agent is cholesterol or derivative thereof.

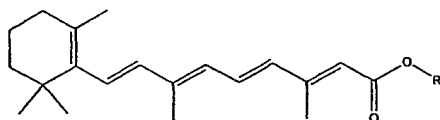
10 Another embodiment of the present invention is a use of a composition as described above,
 wherein said cholesterol derivatives are any of cholesteryl-3-betahydroxybutyrate,
 cholesteryl-halogenated butyrate, cholesteryl-halogenated acetate, cholesteryl-halogenated
 aceto-acetate, cholesteryl-halogenated acetamide, cholesteryl-halogenated crotonate,
 cholesteryl-halogenated acetone, cholesteryl-halogenated citrate, or cholesteryl-halogenated
 15 oleate

Another embodiment of the present invention is a use of a composition as described above,
 wherein solubilising agent is vitamin A or derivative thereof.

20 Another embodiment of the present invention is a use of a composition as described above,
 wherein derivative of vitamin A is formula (IV) or (V):



(IV)



(V)

wherein R is selected from the group consisting of betahydroxybutyrate, halogenated
 butyrate, halogenated acetate, halogenated aceto-acetate, halogenated acetamide,
 25 halogenated crotonate, halogenated acetone, halogenated citrate, and halogenated oleate.

Another embodiment of the present invention is a use of a composition as described above, wherein at least one of said inhibitors is present in micro-capsule and/or nano-capsule.

- 5 Another embodiment of the present invention is a use of a composition as described above, wherein nano-capsule is any of copolymer poly(ethylene oxide) with poly(L-Lactic acid) or with poly(beta-benzyl-L-aspartate), copolymer with poly(lactide-co-glycolide)-[(propylene oxide)-poly(ethylene oxide)], polyphosphazene derivatives, poly(ethylene glycol) coated nanospheres, poly(isobutylcyanoacrylate) nanocapsules, poly(gamma-benzyl-L-
10 glutamate)/(poly(ethylene oxide), chitosan-poly(ethylene oxide) nanoparticles, nanoparticles where said inhibitor is prepared using o-carboxymethylate chitosan as wall forming material, or solid lipid nanospheres (SLN).

- Another embodiment of the present invention is a use of a composition as described above, wherein micro-capsule is any of multiporous beads of chitosan, coated alginate
15 microspheres, N-(aminoalkyl) chitosan microspheres, chitosan/calcium alginate beads, poly(adipic anhydride) microspheres, gellan-gum beads, poly(D, L-lactide-co-glycolide) microspheres, alginate-poly-L-lysine microcapsules, crosslinked chitosan microspheres, chitosan/gelatin microspheres, crosslinked chitosan network beads with spacer groups, 1,5-
20 dioxepan-2-one microspheres, D,L-dilactide microspheres, triglyceride lipospheres, polyelectrolyte complexes of sodium alginate chitosan, polypeptide microcapsules, or albumin microspheres.

- Another embodiment of the present invention is a use of a composition as described above, wherein said composition is part of a solid wall composition.
25

- Another embodiment of the present invention is a use of a composition as described above, wherein said solid wall composition is a capsule of suitable size and shape for administration using a needle, said capsule filled with composition.
30

Another embodiment of the present invention is a use of a composition as described above, wherein a wall of said capsule comprises gelatin.

Another embodiment of the present invention is a use of a composition as described above, wherein said solid wall composition is a solid state bioabsorbable structure of suitable size and shape for administration using a needle, said structure impregnated with composition.

- 5 Another embodiment of the present invention is a use of a composition as described above, wherein said solid state bioabsorbable structure is seed-shaped, rod-shaped, or tube-shaped.

- Another embodiment of the present invention is a use of a composition as described above,
10 further combined with radiotherapy.

Another embodiment of the present invention is a use of a composition as described above, further combined with chemotherapy.

- 15 Another embodiment of the present invention is a use of a composition as described above, wherein said composition is administered by injection into a mass of proliferating the cells.

- Another embodiment of the present invention is a use of a composition as described above, wherein said composition is administered by infusion into a mass of the proliferating cells.
20

Another embodiment of the present invention is a use of a composition as described above, wherein said composition is administered by high-pressure injection into a mass of the proliferating cells.

- 25 Another embodiment of the present invention is a use of a composition as described above, wherein said composition is administered in the resection cavity or scar of a mass of the proliferating cells.

- Another embodiment of the present invention is a kit comprising a composition comprising
30 one or more inhibitors of the TCA cycle and/or one or more inhibitors of glycolysis.

Another embodiment of the present invention is a kit as described above, wherein said composition is a composition as defined above.

Another embodiment of the present invention is a kit as described above, further comprising a syringe.

Another embodiment of the present invention is a use of a composition as defined above
5 wherein a inhibitor of TCA cycle and said proliferating cells are dysplasia of the cervix uteri.

Another embodiment of the present invention is a hydrogel comprising a) composition as defined above, and
b) an activated polyethyleneglycol (PEG) combined with any of alkaline hydrolyzed soya
10 solutions, animal or vegetal proteins, bovine serum albumin, soya globulin, casein, pea albumin, starch albumine, or ovalbumin.

Another embodiment of the present invention is a hydrogel as defined above wherein a TCA inhibitor of the composition is present at a concentration of less than or equal to 0.1 mg per
15 square cm of hydrogel and/or a glycolysis inhibitor of the composition is present at a concentration of less than or equal to 10 mg per square cm of hydrogel.

Another embodiment of the present invention is a use of a hydrogel as described above for treatment of superficial cell proliferation, such as basal carcinoma or a squamous cell
20 carcinoma by application of the hydrogel to the surface of said proliferations.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of substances that block the citric acid cycle (TCA cycle) and optionally glycolysis, for the treatment of the proliferation of cells.
25

Proliferative or proliferating cells, whether benign or malignant, are cells such cancer cells, vascular restenosis cells, hypertrophic scar cells, cheloid cells, inflammatory cells, benign tumor cells or any rapidly proliferating cell. Such cells are found in, for example, tumours, hyperproliferative scars, cheloid scars, myoma or fibroma benign tumors and restenotic
30 processes or tumors at the level of a duct. Generally such cells rapidly proliferate (hyperproliferative). A collection of such cells form a cell mass.

Magnetic resonance (MR) spectroscopy studies and positron emission tomography PET-CT studies performed by the inventors have shown the TCA cycle is more active or highly active
35 in a majority of hyperproliferative cells, compared with adjacent cells not undergoing

hyperproliferation. This property enables one or more TCA cycle inhibitors to be employed proximal to the site of cell proliferation, and to be rapidly taken up by said proliferating cells in doses higher than by non-proliferating cells. Consequently high doses of inhibitor or extremely potent inhibitors may be applied locally, resulting in cell death of hyperproliferative cells, and reduced or no cell death of non-proliferating cells. The high local concentration of inhibitors reached by the intra-lesional injection and the difference in TCA metabolism between proliferating and non proliferating cells can allow for a reduction in the amount of active substance necessary compared with conventional chemotherapy delivered by intravenous route.

10

Furthermore, the inventors have realised that inhibition of one or more enzymes in the TCA cycle alone can lead to cell death. The inventors have also found that even further cell death is achieved when glycolysis is additionally inhibited.

15

One embodiment of the present invention is a use of a composition comprising one or more inhibitors of the TCA cycle and optionally one or more inhibitors of glycolysis for treating hyperproliferative cells administered to or in the region of the proliferation, preferably into the proliferating cell mass.

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One embodiment of the present invention is a use of a composition comprising one or more inhibitors of the TCA cycle and optionally one or more inhibitors of glycolysis for the preparation of a medicament for treating hyperproliferative cells, wherein the composition is administered to or in the region of the proliferation, preferably into the proliferating cell mass.

25

One embodiment of the present invention is a method for the treatment of cellular proliferation comprising administering to a patient a composition comprising one or more inhibitors of the TCA cycle and optionally one or more inhibitors of glycolysis for treating hyperproliferative cells, said administration to or in the region of the proliferation, preferably into the proliferating cell mass.

30

Another embodiment of the present invention is a use of a composition comprising one or more inhibitors of the TCA cycle, one or more inhibitors of glycolysis, and one or more slow release agents for the preparation of a medicament for treating hyperproliferative cells, wherein the composition is administered to or in the region of the proliferation, preferably into

35

the proliferating cell mass.

The present invention also relates to a method for sensitising proliferating cells present in a cavity of a subject to treatment by radiotherapy and/or chemotherapy, comprising administering into the proliferating cell mass a composition comprising one or more inhibitors of the TCA cycle and/or one or more inhibitors of glycolysis prior to said radiotherapy and/or chemotherapy.

The present invention also relates to the use of substances that inhibit glycolysis, for the treatment of the proliferation of cells.

One or more glycolysis inhibitors may be employed proximal to the site of cell proliferation, to be rapidly taken up by proliferating cells in doses higher than by non-proliferating cells. Consequently high doses of inhibitor or extremely potent inhibitors may be applied locally, resulting in cell death of hyperproliferative cells, and reduced or no cell death of non-proliferating cells. The differential in glycolysis metabolism can allow for a reduction in the amount of active substance necessary compared with conventional chemotherapy.

One embodiment of the present invention is a use of a composition comprising one or more inhibitors of glycolysis for treating hyperproliferative cells administered to or in the region of the proliferation, preferably into the proliferating cell mass..

One embodiment of the present invention is a use of a composition comprising one or more inhibitors of glycolysis for the preparation of a medicament for treating hyperproliferative cells, wherein the composition is administered to or in the region of the proliferation, preferably into the proliferating cell mass..

One embodiment of the present invention is a method for the treatment of cellular proliferation comprising administering to a patient a composition comprising one or more inhibitors of glycolysis for treating hyperproliferative cells, said administration to or in the region of the proliferation, preferably into the proliferating cell mass..

The composition can be a pharmaceutical composition. Where a particular use of a composition of the present invention is described, said use may be understood as a method. In another preferred mode of the invention an inhibitor of the TCA cycle is fluoroacetate. In another preferred mode of the invention an inhibitor of glycolysis is 2FDG (2-deoxy-2-fluoro-D-glucose). In another preferred mode of the invention a slow release agent is

polyorthoester. The present invention may be applied using any inhibitors of these pathways as indicated below. The composition may be administered to or in the region of the proliferation. Thus, composition is therefore, administered locally to the site of proliferation, and not systemically. Preferably it is administered into the proliferating cell mass.

5

TCA cycle inhibitors

A TCA cycle inhibitor of the invention is any inhibitor of one or more enzymes of the TCA cycle. The TCA cycle enzymes are known in the art and include pyruvate dehydrogenase, citrate synthase, aconitase, isocitrate lyase, alpha-ketoglutarate dehydrogenase complex, succinyl CoA synthetase, succinate dehydrogenase, fumarase, malate synthase, glutaminase, and pyruvate dehydrogenase complex. It is an aspect of the invention that an inhibitor of aerobic ATP synthesis is an inhibitor of an enzyme associated with the TCA cycle. Inhibitors of the TCA cycle are any known in the art.

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The availability of reduced and oxidized forms of nicotinamide adenine dinucleotide (NAD⁺ and NADH) is important for the TCA and depletors of NAD⁺ and NADH would be inhibitors of the TCA cycle. Depletors of NAD⁺ and/or NADH include Hypoglycin A and its metabolite methylenecyclopropylacetic acid, ketone bodies (D(-)-3-hydroxybutyrate), alloxan, PNU and any other substance known in the art.

20

Inhibitors of pyruvate dehydrogenase are any known in the art and may include, but are not limited to any of arsenite, dichlorovinyl-cysteine, p-benzoquinone, thiaminase.

25 Inhibitors of citrate synthetase are any known in the art and may include, but are not limited to any of the following:

Fluoroacetate (an its derivative fluoroacetyl-CoA), any halogenated acetyl-CoA, fluoroacetamide, fluorocrotonate, halogenated ketone bodies (for instance, chloroacetoacetate, fluoroacetoacetate, fluorohydroxybutyrate, chlorohydroxybutyrate, bromohydroxybutyrate), halogenated acetone, halogenated acetic acid (for example chloroacetic acid), halogenated oleate (an analogue of ketone bodies) and any known in the art.

30

Inhibitors of aconitase are any known in the art and may include, but are not limited to any of the following:

35

Fluorocitrate, fluorocitrate 2R, 3R, and any other halogenated citrate (bromocitrate, chlorocitrate).

- Inhibitors of isocitrate dehydrogenase are any known in the art and may include, but are not limited to any of the following:
- 5 DCVC (dichlorovinyl-cysteine)

- Inhibitors of succinate dehydrogenase are any known in the art and may include, but are not limited to malonate, DCVC, Pentachlorobutadienyl-cysteine (or PCBD-cys), 2-bromohydroquinone, 3-nitropropionic acid, cis-crotonalide fungicides.
- 10

Inhibitors of succinyl CoA synthetase, alpha ketoglutarate dehydrogenase complex, fumarate hydratase (fumarase), or malate dehydrogenase are any known in the art.

- 15 Inhibitors of glutaminase are any known in the art and may include, but are not limited to 6-diazo-5-oxo-L-norleucine (DON).

Inhibitors of glutamate dehydrogenase are any known in the art.

- 20 Other inhibitors of the TCA cycle include glu-hydroxyoxamate, p-chloromercuriphenylsulphonic acid (impermeant thiol agent), L-glutamate gamma-hydroxamate, p-chloromercuriphenylsulphonic acid, acivicin (alpha-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid), halogenated glutamine and glutamate.

- 25 Several other compounds may block the production of ATP at the level of the TCA cycle after compound transformation. Indeed, most amino-acids may be degraded to enter the TCA cycle at various places. Therefore, most of aminoacids used in an halogenated formulation will be able to block the TCA cycle by being degraded to one of the TCA products (halogenated glutamate, glutamine, histidine, proline, arginine, valine, methionine, threonine, isoleucine, aspartate, tyrosine, phenylalanine, asparagine, aspartate, alanine, glycine, cysteine, serine, threonine). Some of the amino-acids will be transformed into ketone bodies. These amino-acids (leucine, lysine, phenylalanine, tyrosine) in an halogenated presentation will interact at the same sites where halogenated ketone bodies interact as described previously. Finally, some amino-acids in an halogenated formulation (tryptophan, leucine,
- 30

isoleucine) will be directly transformed into acetyl-CoA and will block the TCA at the level of citrate synthase - aconitase.

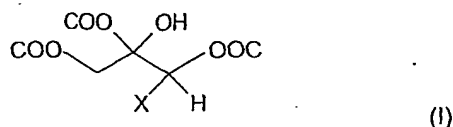
According to another embodiment of the invention, a TCA cycle inhibitor is any of arsenite, hypoglycin A, methylenecyclopropylacetic acid, alloxan, PNU, p-benzoquinone, fluoroacetate, halogenated acetates (iodo-, bromo-, chloro-acetate), halogenated acetyl-CoA (fluoroacetyl-CoA, bromoacetyl-CoA, chloroacetyl-CoA, iodoacetyl-CoA), halogenated crotonate (fluoro-, iodo-, bromo-, chloro-crotonate), halogenated ketone bodies, (chloro-, fluoro-, bromo-, iodoaceto-acetate, fluoro-, chloro-, bromo-, iodo-butyrate, fluoro-, chloro-, bromo-, iodo-acetone), halogenated oleate (iodo, bromo, chloro, fluoro-oleate), halogenated citrate, halogenated citrate 2R, 3R isomer (fluoro-, bromo-, chloro-, iodo-citrate), dichlorovinyl-cysteine, halogenated aminoacids, malonate, pentachlorobutadienyl-cysteine, 2-bromohydroquinone, 3-nitropropionic acid, cis-crotonalide fungicides, glu-hydroxyoxamate, p-chloromercuriphenylsulphonic acid, L-glutamate gamma-hydroxamate, p-chloromercuriphenylsulphonic acid, acivicin (alpha-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid), halogenated glutamine (fluoro, iodo, chloro, bromo-glutamine), or halogenated glutamate (fluoro, iodo, chloro, bromo-glutamate).

Where more than one inhibitor of the TCA is present in a composition, preferably, one inhibitor is directed towards the upper half of the TCA cycle, which is characterised by providing no redox products such as NADH, HANPH, or FADH₂ (e.g. enzymes pyruvate dehydrogenase, citrate synthase, aconitase) and another inhibitor is directed towards the lower half of the TCA cycle, which is characterised by providing redox products such as NADH, HANPH, or FADH₂ (e.g. enzymes isocitrate lyase, alpha-ketoglutarate dehydrogenase complex, succinyl CoA synthetase, succinate dehydrogenase, malate synthase, glutaminase). Examples of a combination of inhibitor includes fluoroacetate and malonate.

Fluorocitrate and derivatives

According to a preferred embodiment of the invention, a TCA cycle inhibitor of the invention has a formula (I):

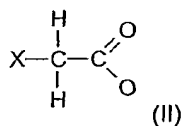
16



where X may be halide, a sulfonate, a carboxylate, an alkoxide, an amine oxide or a OH. The
 5 halide may be selected from the group consisting of: fluoride, bromide, chloride, and iodide.
 The sulfonate may be selected from the group consisting of: triflate, mesylate and tosylate.
 The carboxylate may be selected from the group consisting of: methoxylate and ethyloxylate.
 The alkoxide may be selected from the group consisting of: methoxide and ethoxide. The
 amine oxide is dimethylamine oxide. According to one aspect of the invention, the
 10 stereochemistry is 2R, 3R.

Fluoroacetate and derivatives

TCA cycle inhibitors also includes substances which are converted into inhibitors of the TCA
 cycle such as, for example fluoroacetate and derivatives. According to a preferred
 15 embodiment of the invention, a TCA cycle inhibitor of the invention has a formula (II):



where X may be halide, a sulfonate, a carboxylate, an alkoxide, or an amine oxide, a OH.
 20 The halide may be selected from the group consisting of: fluoride, bromide, chloride, and
 iodide. The sulfonate may be selected from the group consisting of: triflate, mesylate and
 tosylate. The carboxylate may be selected from the group consisting of: methoxylate and
 ethyloxylate. The alkoxide may be selected from the group consisting of: methoxide and
 ethoxide. The amine oxide may be dimethylamine oxide.

25

Preferably, an inhibitor of the TCA cycle is any of fluoroacetate, fluorocitrate, arsenite,
 acetoacetate, and betahydroxy butyrate.

30 *Halogenated inhibitors which are radioisotopes*

According to an aspect of the present invention, at least one halogen atom where present in an inhibitor of the TCA cycle is substituted for the corresponding halogen atom radioisotope, to form a radio-isotope-halogen TCA cycle inhibitor (RIH-TCA cycle inhibitor). The radioisotopes of halides may be for example ^{18}F , ^{79}Br , ^{81}Br , ^{36}Cl , ^{125}I , ^{129}I , ^{131}I , which all emit ionising radiation. For example, the stable fluorine atom of fluoroacetate may be substituted for ^{18}F to form ^{18}F -fluoroacetate. Similarly ^{125}I -iodoacetate may be employed as a TCA cycle inhibitor. The use of an RIH-TCA cycle inhibitor allows effective brachytherapy, simultaneously with pathway inhibition as described above. Furthermore, where used in combination therapy the RIH-TCA cycle inhibitor can be administered sequentially after an oxidative phosphorylation and/or glycolysis inhibitor; the proliferating cells, therefore, are affected both in terms of energy production by the inhibitors and by the ionising radiation of the RIH-TCA cycle inhibitor. The dose of the RIH-TCA cycle inhibitor can be adjusted so that the cytotoxic effect is due to the ionising radiation rather than pathway inhibition, or *vice versa*.

15

Simultaneous inhibition of other pathways

According to an aspect of the invention a TCA cycle inhibitor is capable of inhibiting at least 3 cellular mechanisms of proliferating cells simultaneously. This may be achieved by blocking, for example, aconitase from the TCA cycle. The inventors have realised that the use of an aconitase inhibitor such as, for example, fluorocitrate (of fluoroacetate which is later converted into fluorocitrate) can inhibit other important pathways such as fatty acid synthesis at the level of ATP-citrate lyase and calcium intracellular signalling through derivatives accumulation.

20

Glycolysis inhibitors

According to one embodiment of the invention, an inhibitor of glycolysis inhibits at least one enzyme from the group consisting of hexokinase, glucokinase, phosphoglucose isomerase, phosphofructokinase, aldolase, triose phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglyceromutase, enolase, pyruvate kinase, and lactate dehydrogenase.

25

According to another embodiment of the invention, an inhibitor of glycolysis is a hexose sugar modified by removal of the hydroxyl group or by the substitution of the hydroxyl group with halogen atom or thiol at:

30

- C6 for inhibiting hexokinase,
- C1 or C2 or C5 for inhibiting phosphoglucoisomerase
- C3 and/or C4 for blocking aldolase, and/or
- C2 or C3 for blocking glyceraldehyde 3P deshydrogenase, phosphoglycerate kinase,
- 5 phosphoglycerate mutase, and enolase.

According to another embodiment of the invention, an inhibitor of glycolysis is any of 6-deoxy-6-fluoro-D-glucose, 6-deoxy-6-bromo-D-glucose, 6-deoxy-6-chloro-D-glucose, 6-O-methyl-D-glucose, 6-thio-D-glucose, 6-deoxy-D-glucose, C-6 modified or blocked derivatives

10 of other hexose ring pyranoses, mannopyranoses, galactopyranoses, 6-deoxy-6-fluoro-D-glucose, 6-deoxy-6-bromo-D-mannose, 6-deoxy-6-chloro-D-mannose, 6-deoxy-6-fluoro-D-galactose, 6-deoxy-6-chloro-D-galactose, 6-deoxy-6-iodo-D-galactose, 6-deoxy-6-bromo-D-galactose, halogenated C-6 sugars gluconolactones, glucuronic acid, glucopyranoside, and their phosphate derivatives, glucoronides with halogenated glycosides at the C-1 position, C-

15 2 substituted D-hexoses, 2-deoxy-2-halogeno-D-hexoses, 2-deoxy-2-fluoro-D-glucose (2FDG), 2-chloro-2-deoxy-D-glucose, 2-bromo-D-glucose, 2-iodo-D-glucose, 2-deoxy-2,2-difluoro-D-arabino-hexose, 2-deoxy-2-fluoro-D-mannose, 2-deoxy-D-arabino-hexose, 2-Deoxy-2-fluoro-D-galactose, 1,6-anhydro-2-deoxy-2-fluoro-beta-D-glucopyranose, 1-6-anhydrosugar, 2-amino-2-deoxy-D-glucose, glucose amine, 2-amino-2-deoxy D-galactose,

20 galactosamine, 2-amino-2-deoxy-D-mannose, mannosamine, 2-deoxy-2-fluoro-D-mannose, 2-deoxy-2-fluoro-D-galactose, 2-deoxy-D-arabino-hexose, 2-deoxy-2,2-difluoro-D-arabino-hexose, 2-deoxy-2-fluoro-D-glucose 1-Phosphate, 2-deoxy-2-fluoro-D-glucose 6-P, 2-deoxy-2-fluoro-D-glucose 1,6 biphosphate, 2-deoxy-2-fluoro-D-mannose 1-P, 2-deoxy-2-fluoro-D-mannose 6-P, 2-deoxy-2-fluoro-D-mannose 1,6-biphosphate, nucleotide diphosphate, uridine

25 di-P, 1-2 deoxy-2-fluoro-D-glucose, C-2-halogen substituted, and NH₃ substituted derivatives of D-Glucose 6-phosphate, 2-deoxy-2-fluoro-2-D-glucose-6-phosphate, 2-chloro-2-deoxy-D-glucose-6-phosphate, 2-deoxy-D-arabino-hexose-6-phosphate, D-glucosamine-6-phosphate, 2-deoxy-2-fluoro-2-D-manose-6-P, and any known derivatives, C-2 halogenated derivatives of hexose ring pyranoses, mannopyranoses, galactopyranoses, C-2-deoxy-2-

30 fluoropyranoses, and any derivative, C-2 halogenated sugars derivatives, C-2 fluoro-, bromo-, chloro-, or iodo-sugars derivatives, fluoro, bromo, chloro, or iodo C-2 sugars derivatives, gluconolactones, glucuronic acid, glucopyranoside, and their phosphate derivatives, sugars modified at C-1 or C-5 by replacement of hydroxyl by fluorine or deoxygenation or replacement by a sulfur group, glucosyl fluoride, 1-deoxy-D-glucose, 5-thio-D-glucose, 3-

35 deoxy or 3-fluoro-D-glucose or 4-deoxy or 4-fluoro-D-glucose, 2-fluoro- or 2-iodo-, or 2-thio-,

or 2-methoxy-, or 3-fluoro-, or 3, 3 difluoro-, 3-iodo-, or 3-carboxylo-, or 3-thio-glyceraldehydes or glycerates, 3-fluoro-2-phosphoglycerate, phosphothioesters or other phosphor modified analogs, mannoheptulose mannoheptose, glucoheptose, N-acetylglucosamine, 6-aminonicotinamide acidosis-inducing agents, 2-deoxy-2-fluoro-D-
 5 glucose, citrate and halogenated derivatives of citrate, fructose 2,6-bisphosphate, bromoacetyethanolamine phosphate analogues, N-(2-methoxyethyl)-bromoacetamide, N-(2-ethoxyethyl)-bromoacetamide, N-(3-methoxypropyl)-bromoacetamide, iodoacetate, pentalenolactone, arsenic, 1,1-difluoro-3-phosphate-glycerol, oxamate, 2-fluoro-propionic acid or it salts, 2,2-difluoro-propionic acid, 3-halopropionic acid, and 2-thiomethylacetic acid.

10

Preferably, an inhibitor of glycolysis is any of 2FDG, oxamate or iodoacetate.

Glycolysis is the main the pathway for anaerobic ATP synthesis. Tumours switch to anaerobic ATP synthesis by metabolising the well-distributed glucose among others in order
 15 to provide nucleotides through the PPP pathway. It is known that proliferating masses which are partly under anaerobic type respiration are more resistant to radiation or chemotherapy. Therefore, by locally inhibiting the glycolysis pathway, anaerobic respiration which is the principal energy pathway of poorly oxygenated cells is inhibited, leading to increased cell death of hypoxic proliferating cells. The proliferation of non-hypoxic cells is slowed as well
 20 owing to the shutdown of this primary energy pathway.

Halogenated inhibitors which are radioisotopes

According to an aspect of the present invention, at least one halogen atom where present in
 25 an inhibitor of glycolysis is substituted for the corresponding halogen atom radioisotope, to form a radio-isotope-halogen glycolysis inhibitor (RIH-glycolysis inhibitor). The radioisotopes of halides may be for example ^{18}F , ^{79}Br , ^{81}Br , ^{36}Cl , ^{125}I , ^{129}I , ^{131}I , which all emit ionising radiation. For example, the stable fluorine atom of 2FDG may be substituted for ^{18}F to form ^{18}F -2FDG. Similarly -6-deoxy-6- ^{129}I -D-galactose may be employed as a glycolysis inhibitor.
 30 The use of an RIH-glycolysis inhibitor allows effective brachytherapy, simultaneously with pathway inhibition as described above. Furthermore, where used in combination therapy the RIH-glycolysis inhibitor can be administered sequentially after an oxidative phosphorylation and/or glycolysis inhibitor; the proliferating cells, therefore, are affected both in terms of energy production by the inhibitors and by the ionising radiation of the RIH-glycolysis

inhibitor. The dose of the RIH-glycolysis inhibitor can be adjusted so that the cytotoxic effect is due to the ionising radiation rather than pathway inhibition, or *vice versa*.

Simultaneous, sequential and separate

- 5 According to one aspect of the invention, a glycolysis inhibitor may be administered simultaneous, separate or sequentially in respect of a TCA cycle inhibitor of the invention.

Another aspect of the invention is a composition comprising at least one TCA cycle inhibitor as disclosed herein and at least one glycolysis inhibitor, for simultaneous, separate or
10 sequential administration to a subject.

One aspect of the invention is a method for treating cellular proliferation comprising administering to an individual an effective amount of at least one TCA cycle inhibitor of the invention and at least one glycolysis inhibitor, simultaneously, separately or sequentially.
15

By simultaneous administration means the TCA cycle inhibitor and glycolysis inhibitor are administered to a subject at the same time. For example, as a mixture or a composition comprising said components. An example is as a solution comprising the components of interest.
20

By separate administration means the TCA cycle inhibitor and glycolysis inhibitor are administered to a subject at the same time or substantially the same time. The components may be present in a kit as separate, unmixed preparations. For example, the TCA cycle inhibitor and glycolysis inhibitor may be present in the kit as individual vials. The inhibitors
25 may be administered to the subject by separate injections at the same time, or injection directly following the other.

For example, in a prostate cancer, 12 punctures may be performed in the peripheral area of the prostate. One out of each second puncture is made with a composition comprising a slow release formulation of fluoroacetate, and the other puncture is performed with a composition
30 comprising a slow release formulation of oxamate. Injection sessions may be repeated several times, depending on PSA levels.

In another example, a slow release formulation of a TCA inhibitor may be injected inside a proliferative process such as a tumor, and a preparation of glycolysis inhibitors may be delivered through an infusion at the same time or a few days later.

- 5 Both therapeutic substances may also be mixed in a same slow release formulation. It is also possible to administer one formulation first, and the other formulation later on, if PSA levels do not come down completely after the first therapy.

10 By sequential administration means the TCA cycle inhibitor and glycolysis inhibitor are administered to a subject sequentially. The TCA cycle inhibitor and glycolysis inhibitor may be present in a kit as separate, unmixed preparations. There is a time interval between doses. For example, one component might be administered up to 336, 312, 288, 264, 240, 216, 192, 168, 144, 120, 96, 72, 48, 24, 20, 16, 12, 8, 4, 2, 1, or 0.5 hours after the other component.

15 Taking the above mentioned example again, the 12 punctures around a prostate cancer may first be made with a composition comprising a slow release formulation of fluoroacetate. Later on other punctures are performed with a composition comprising a slow release formulation of oxamate, if PSA levels do not come down completely after the first therapy.

20 In sequential administration, one component may be administered once, or any number of times and in various doses before and/or after administration of another component. Sequential administration may be combined with simultaneous or sequential administration.

25 *Derivatives*

Stereoisomer, tautomers, racemates, prodrugs, metabolites, pharmaceutically acceptable salts, bases, esters, structurally related compounds or solvates of TCA cycle or glycolysis inhibitors are within the scope of the invention.

30 The pharmaceutically acceptable salts of the compounds according to the invention, *i.e.* in the form of water-, oil-soluble, or dispersible products, include the conventional non-toxic salts or the quaternary ammonium salts which are formed, *e.g.*, from inorganic or organic acids or bases. Examples of such acid addition salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate,

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fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as sarginine, lysine, and so forth. Also, the basic nitrogen-containing groups may be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl; and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl-bromides and others. Other pharmaceutically acceptable salts include the sulfate salt ethanolate and sulfate salts.

The term "stereoisomer", as used herein, defines all possible compounds made up of the same atoms bonded by the same sequence of bonds but having different three-dimensional structures which are not interchangeable, which the compounds of the present invention may possess. Unless otherwise mentioned or indicated, the chemical designation of a compound herein encompasses the mixture of all possible stereochemically isomeric forms, which said compound may possess. Said mixture may contain all diastereomers and/or enantiomers of the basic molecular structure of said compound. All stereochemically isomeric forms of the compounds of the invention either in pure form or in admixture with each other are intended to fall within the scope of the present invention.

The compounds according to the invention may also exist in their tautomeric forms. Such forms, although not explicitly indicated in the compounds described herein, are intended to be included within the scope of the present invention.

For therapeutic use, the salts of the compounds according to the invention are those wherein the counter-ion is pharmaceutically or physiologically acceptable.

As used herein and unless otherwise stated, the term "solvate" includes any combination which may be formed by a compound of this invention with a suitable inorganic solvent (e.g. hydrates) or organic solvent, such as but not limited to alcohols, ketones, esters and the like.

The term "pro-drug" as used herein means the pharmacologically acceptable derivatives such as esters, amides and phosphates, such that the resulting *in vivo* biotransformation product of the derivative is the active drug. The reference by Goodman and Gilman (The Pharmacological Basis of Therapeutics, 8th Ed, McGraw-Hill, Int. Ed. 1992, "Biotransformation of Drugs", p 13-15) describing pro-drugs generally is hereby incorporated.

5 Pro-drugs of the compounds of the invention can be prepared by modifying functional groups present in said component in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent component. Typical examples of pro-drugs are described for instance in WO 99/33795, WO 99/33815, WO 99/33793 and WO 99/33792 all incorporated herein by reference. Pro-drugs are characterized by increased bio-availability and are readily metabolized into the active inhibitors *in vivo*. Specific examples of prodrugs comprising cholesterol or vitamin A are described below.

15 Administration

Administering to the region of the proliferation avoids or minimises peripheral toxicity, and permits delivery of efficacious doses. It also avoids toxicity associated with oral or systemic delivery. Methods of local delivery are known in the art.

- 20 According to one embodiment of the invention, the composition is administered into the proliferating tissue. The composition can enter the tissue, for example, by puncturing the surface of the tumour and injecting composition therein. Such administration is achieved by positive pressure application, for example, by high or low pressure injection, etc as described below. Alternatively, it may be achieved by intra-arteria infusion as also described below.
- 25 Alternatively, the composition may enter the tissue after opening, for example, by resection surgery to remove the proliferating cell mass, as elaborated below.

- According to one embodiment of the invention, a composition is administered under positive pressure to a proliferating tissue. Devices suitable for pressured delivery are known in the art, for example from US 2001/0034503 which is incorporated herein by reference. The latter document describes an apparatus for injecting a composition into a tissue under high pressure through a thin catheter (200 microns outer diameter for instance) with very small lateral holes (50 microns) located at the level of the tissue. One may leave the catheter
- 30 inside the proliferating tissues for several days, and inject determined amounts of
- 35

substances directly inside the proliferating structure at regular intervals allowing effective penetration of tissue. Where the proliferating cells form a large tissue mass, for example, a pressure between 2 to 5000 Atm. may used to apply the composition, leading to effective distribution of several centimetres radius within the tissue mass. Such injections are rapidly administered in a single shot. As the injected substances may be very toxic (fluorocitrate, arsenate, cyanide), it is important to be able to modulate the injected amounts very precisely as it is the case with this device. According to an aspect of the invention, a quantity of composition between 1 microlitre and 100 ml is injected at a pressure between 100 and 5000 Atm. The treatment may be repeated at defined intervals or as necessary.

10

According to another embodiment of the invention, a composition is delivered locally, preferably into the proliferating cell mass, under pressure by means of a continuous pump. A pump may be used to inject composition over a period of time, from several hours to several days as necessary. A pump may take the form of a mechanically operated syringe.

15

According to another embodiment of the invention a composition is delivered intra-arterially using protracted infusion. Use of intra-arterial protracted infusion allows the composition to infuse directly into the proliferating structure, via a feeding artery. A catheter may be left in place for several minutes to months in order to deliver the therapy efficiently.

20

According to one embodiment of the invention, a composition is administered under positive pressure to a proliferating tissue by directed injection into the tissue, for example using a needle and syringe.

25

According to another embodiment of the present invention, the composition is administered by injection or deposition into a resection cavity or scar or area of proliferating cells. Thus resection cavities after surgical debulking of tumours can be treated. For example, said cavity any of brain tumour resection, breast tumour resection, prostate cancer resection, muscle resection after a sarcoma, uterine laparoscopic myoma resection, head and neck resection cavities, tongue tumour resection, partial upper maxillar resection, liver tumour resection, kidney tumour resection, or bone tumour resection, scar cavity of a melanoma resection. Furthermore, the bed of cheloid scars after resection may be treated by the present composition, in order to avoid cheloid or hypertrophic scar formation in the population known at risk for such reactions.

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According to another embodiment of the present invention, said composition is injected into a mass of proliferating cells such as a tumour under visual control, using ultrasound, MRI, CT-scan, PET-CT or any other imaging means. The authors have found that intratumour diffusion is optimal when there is an homogeneous coverage of the whole tumour volume, and that an injection performed tangentially to the tumour volume is less efficacious. Such mode of injection may be performed using a syringe, pump or high pressure as described above.

Administering a composition of the present invention in a proliferating cell mass not only treats the cell mass but also the lymphatic system through which the tumour drains. With conventional therapies, the such as radiotherapy the lymphatic pathways are destroyed. With systemic chemotherapy, there is no selective treatment of the lymphatic pathways. In the present invention, by contrast, both the lymphducts and lymph nodes are selectively treated, in addition to the cellular proliferation.

15

- Slow release formulation

According to another embodiment of the invention, a slow release formulation of the composition is delivered by application, injection or puncture to or proximal to the site of proliferation. Preferably it is administered into a mass of proliferating cells. The slow release agent regulates, slows the release of inhibitor from the composition. A single dose can comprise a large or concentrated dose, which, once at the site of proliferation, is released at a rate determined by the formulation. This avoids the need for prolonged treatment times associated with other delivery methods such as infusion or positive pressure. It also avoids the need for equipment to deliver doses under high pressure and the frequency of administration associated therewith. Another advantage of a slow release formulation is that the composition diffuses day and night, over several days or weeks. The inhibitors can act when a patient is fasting, (e.g. every night) and there is no competition from the degradation products of ingested meals.

25
30

The present inventors have found that inhibitor uptake can be relatively slow in some tumours by observing ^{18}F -FDG and ^{11}C -acetate by PET-CT. Although tumours may show as an intense signal due to the sensitivity of the imaging and probe, this is deceptive of the uptake rate which can be relative low e.g in the range of ng/min. Consequently, a slowly

releasing inhibitor is better able to match the rate of inhibitor take by the tumour, and avoid wasteful and toxic overdosing.

For instance, a slow release formulation of fluoroacetate may be applied on a cervical area, if
5 a cervical dysplasia is diagnosed. Precancerous pathologic areas (dysplasia) are usually detected under colposcopy (enlarged view of the cervix using a camera) using acetate applied on the cervix, with dysplastic areas indicating a colour change after this application (withening). In the present case, fluoroacetate in a slow release formulation (e.g. polyorthoester polymer) adheres to the cervix and is actively absorbed by the transformed
10 cells, which actively absorb acetate during diagnostic colposcopy. The dysplastic cells are destroyed, avoiding the need for a superficial laser therapy or a limited cervical excision. The cervical treated area may be covered by a mechanical structure such as a diaphragm, which prevents a direct contact between the polymer and the drug with the vagina.

15 One embodiment of the present invention is a composition as described herein, further comprising one or more slow release agents. Slow release agents may be natural or synthetic polymers, or reabsorbable systems such as magnesium alloys.

Among the synthetic polymers useful according to a slow release formulation of the invention
20 are poly(glycolic) acid, poly(lactic acid) or in general glycolic- and lactic acid based polymers and copolymers. They also include poly caprolactones and in general, poly hydroxyl alkanooates (PHAs) (poly(hydroxy alcanoic acids) = all polyester). They also include Poly (ethylene glycol), poly vinyl alcohol, poly (orthoesters), poly (anhydrides), poly (carbonates), poly amides, poly imides, poly imines, poly (imino carbonates), poly (ethylene imines),
25 polydioxanes, poly oxyethylene (poly ethylene oxide), poly (phosphazenes), poly sulphones, lipids, poly acrylic acids, poly methylmethacrylate (PMMA), poly acryl amides , poly acrylo nitriles (Poly cyano acrylates), poly HEMA, poly urethanes, poly olefins, poly styrene, poly terephthalates, poly ethylenes, poly propylenes, poly ether ketones, poly vinylchlorides, poly fluorides, silicones, poly silicates (bioactive glass), siloxanes (Poly dimethyl siloxanes),
30 hydroxyapatites, lactide-capronolactones and any other synthetic polymer known to a person skilled in the art.

Among the natural derived polymers useful according to a slow release formulation of the invention, are poly aminoacids (natural and non natural), poly β -aminoesters. They also
35 include poly (peptides) such as: albumines, alginates, cellulose / cellulose acetates, chitin /

chitosan, collagene, fibrine / fibrinogen, gelatine, lignine. In general, proteine based polymers. Poly (lysine), poly (glutamate), poly (malonates), poly (hyaluronic acids). Poly nucleic acids, poly saccharides, poly (hydroxyalkanoates), poly isoprenoids, starch based polymers, and any other natural derived polymer known to a person skilled in the art.

5

Other polymers may be made from hydrogels based on activated polyethyleneglycols (PEGs) combined with alkaline hydrolyzed animal or vegetal proteins.

For both synthetic and natural polymers, the invention includes copolymers thereof are included as well, such as linear, branched, hyperbranched, dendrimers, crosslinked, functionalised (surface, functional groups, hydrophilic/hydrophobic).

The slow release composition may be formulated as liquids or semi-liquids, such as solutions, gels, hydrogels, suspensions, lattices, liposomes; or implants, such as particles, films, rods, fibres, grains. Solid or semi-solid formulations such as rods, fibres or grains improve the ease of administering the implant. Semi-liquid, liquid substance or polymer, or active substance presenting as powder, may be encapsulated in a resorbable capsule or tube made from gelatine, or any polymer degrading rapidly. Any suitable formulation known to the skilled man is within the scope of the invention. According to an aspect of the invention, a composition is formulated such that the quantity of inhibitor is between less than 1% and 60 % of total slow-release polymer mass. According to an aspect of the invention, a composition is formulated such that the quantity of inhibitor is between 1% and 50%, 1% and 40%, 1% and 30%, 1% and 20%, 2% and 60%, 5% and 60%, 10% and 60%, 20% and 60%, 30% and 60%, or 40% and 60% of total slow-release polymer mass.

25

Where the slow release agent has the properties to form a semi-solid (gel-like) polymer, the composition may take the form of a foil allowing the release of inhibitor in a controlled fashion, for instance in contact with superficial skin cancers. The foil administers composition directly into the cells owing to the properties of the hydrogel; the high water content of the gel creates a fully communicating structure to the interior of the proliferating mass of cells. Oncotic pressure holds water inside hydrogel and attracts waters from skin, and makes easy transfer of molecules from gel to skin.

30

One example of such foil may be, for example, a polymer made from hydrogels based on activated polyethyleneglycols (PEGs) combined with alkaline hydrolyzed soya solutions or other animal or vegetal proteins (bovine serum albumin, soya globulin, casein, pea albumin, starch albumine, ovalbumin, etc).

5

Such a foil could be for instance 3 mm thick and be filled with saline water to a percentage of 90 % or more, containing one to several ATP inhibitors.

Such foils may be used easily to treat superficial skin cancers. For instance, squamous cell skin cancers or basal carcinomas present usually with a round shape, for instance, 1, 3, or 5 cm in diameter. When in contact with the superficial skin tumour, such a hydrogel foil hydrates actively the skin, humidifies the epithelium, and allows easy transfer of inhibitor(s) to the superficial tissues of the tumour.

10 The typical load of such a 3 mm thick hydrogel would be in the range of 10 mg of glycolysis inhibitor per square cm of hydrogel or less and in the range of 0.1 mg of TCA inhibitor per square cm of hydrogel or less.

Once in contact with the lesion, the hydrogel releases slowly the inhibitor(s) inside the superficial tumour.

20

Typically, the major part of each inhibitor will be delivered to the lesion in a period of 4 to 8 hours. The hydrogel may be replaced every day, until the lesion disappears, which should happen within 1 or 2 weeks.

25

The lesion may be treated in addition using radiotherapy. The standard doses for the treatment (typically 10 times 4 Gy) could be reduced by 20 to 50 %.

Solubilising agents

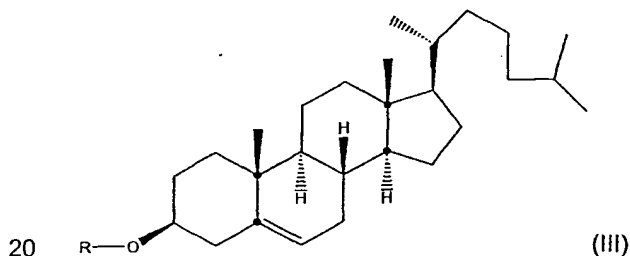
30 According to another embodiment of the present invention at least one TCA cycle inhibitor and/or at least one glycolysis inhibitor of a composition is coupled to one or more solubilising agents. Such agents change the hydrophilic and hydrophobic profile of the inhibitor, depending on the required solubility. For example, if a composition according to the invention comprises a hydrophilic TCA cycle inhibitor such as fluoroacetate, and a hydrophobic slow release polymer such as polyorthoester, the inhibitor will not adequately solubilise or

35

suspend within the composition. Similarly, a composition according to the invention comprising a very hydrophilic glycolysis inhibitor such 2-FDG when placed in a lipophilic slow release agent (polyorthoester), will lead to an inadequately solubilised or emulsified composition. Consequently the release properties of the slow release agent may be compromised, and degradation within the body accelerated. To overcome this, the inventors have coupled at least one TCA cycle inhibitor and/or at least one glycolysis inhibitor to a solubilising agent which changes the hydrophobicity or hydrophilicity of the inhibitor, depending on the required formulation. The composition so formed is more stable. According to one aspect of the invention, the coupled compound is a prodrug wherein the solubilising agent is cleaved *in vivo*, so releasing the inhibitor. According to another aspect of the invention, the solubilising agent is cleaved from the inhibitor more rapidly by the proliferating cells.

- Cholesterol

According to one aspect of the invention, cholesterol (II) or a derivative thereof is a coupling agent. One embodiment of the invention is a composition as mentioned herein in which at least one TCA cycle inhibitor and/or at least one glycolysis inhibitor is coupled to cholesterol (III) or derivatives thereof:



wherein R may be one of the following substances (non exhaustive list):
 betahydroxybutyrate, halogenated butyrate, halogenated acetate, halogenated aceto-
 acetate, halogenated acetamide, halogenated crotonate, halogenated acetone, halogenated
 citrate and halogenated oleate.

25

Derivatives of cholesterol are modifications which retain or enhance of activity of the parent compound. Derivatives include, but are not limited to cholesteryl-3-betahydroxybutyrate, cholesteryl-halogenated butyrate, cholesteryl-halogenated acetate, cholesteryl-halogenated

aceto-acetate, cholesteryl-halogenated acetamide, cholesteryl-halogenated crotonate, cholesteryl-halogenated acetone, cholesteryl-halogenated citrate, or cholesteryl-halogenated oleate.

- 5 Halogenated means fluoro-, chloro-, bromo- or iodo-modified.

An advantage of using cholesterol or a derivative thereof as a solubilising agent is such natural metabolite can enter a cell via a number of mechanisms including through the lipid bilayer of the cell membrane. In rapidly proliferating cells, absorption is more rapid due to the requirement for cholesterol in cell membranes. Once in the lipid bilayer, flippase enzyme transfers the cholesterol-coupled inhibitor from the outer layer to the inner layer; cholesterol is internalised in the cytosol and the inhibitor is released from cholesterol by cholesterol-metabolising enzymes.

- 15 A cholesterol is coupled to an inhibitor using known methods. For example, coupling may proceed via nucleophilic attack by electrons of an oxygen atom on the cholesterol. For example, coupling may proceed via nucleophilic attack by electrons of an oxygen atom on the inhibitor. According to another example, esters, ethers or other derivatives of cholesterol or inhibitor may be prepared to facilitate coupling. Mechanisms and knowledge of appropriate coupling moieties are known to the skilled person for the preparation of such coupled inhibitors.

One embodiment of the present invention is a composition comprising cholesteryl-fluoroacetate and polyorthoester.

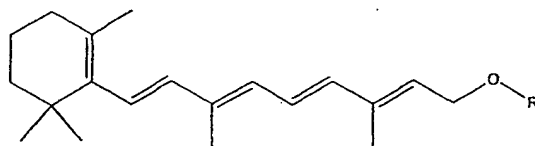
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- Vitamin A

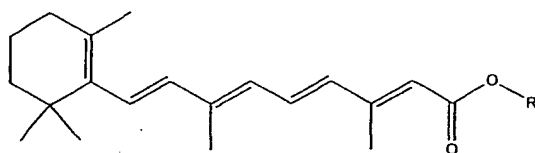
- According to one aspect of the invention, vitamin A (retinol) or a derivative thereof is a coupling agent. One embodiment of the invention is a composition as mentioned herein in which at least one TCA cycle inhibitor and/or at least one glycolysis inhibitor is coupled to vitamin A or derivatives thereof. Examples of derivatives include the ether (IV) and ester (V) forms which groups facilitate ease of coupling:

35

31



(IV)



(V)

Wherein R may be one of the following substances: betahydroxybutyrate, halogenated butyrate, halogenated acetate, halogenated aceto-acetate, halogenated acetamide, halogenated crotonate, halogenated acetone, halogenated citrate, halogenated oleate.

Derivatives of vitamin A are modifications which retain or enhance of activity of the parent compound. Derivatives include, but are not limited to those mentioned above and betahydroxybutyrate, halogenated butyrate, halogenated acetate, halogenated aceto-acetate, halogenated acetamide, halogenated crotonate, halogenated acetone, halogenated citrate, or halogenated oleate.

Halogenated means fluoro-, chloro-, bromo- or iodo-modified.

An advantage of using vitamin A or a derivative thereof as a solubilising agent is that such natural metabolite can enter a cell via a number of mechanisms. In rapidly proliferating cells, absorption is more rapid, especially in vitamin A metabolising cells such as found in liver tissue. The effect may be used to treat, for instance, hepatocarcinomas by injecting a slow release polymer of retinyl ether or retinoic acids ester coupled with haloacetates directly inside the hepatocarcinoma mass. The antiproliferative effect commences once the inhibitor is liberated from the polymer and vitamin A is metabolised.

A vitamin A is coupled to an inhibitor using known methods. For example, coupling may proceed via nucleophilic attack by electrons of an oxygen atom on the vitamin A. For

example, coupling may proceed via nucleophilic attack by electrons of an oxygen atom on the inhibitor. According to another example, esters or other derivatives of vitamin A or inhibitor may be prepared to facilitate coupling. Mechanisms and knowledge of active groups are known to the skilled person for the preparation of such coupled inhibitors

5

One embodiment of the present invention is a composition comprising vitamin A-fluoroacetate and polyorthoester.

Encapsulated inhibitor

- 10 According to one aspect of the invention at least one TCA cycle inhibitor and/or at least one glycolysis inhibitor of a composition is encapsulated in one or more micro-capsules or nano-capsules.

- 15 Examples of nano-capsules (or nano-spheres) or formulations therewith include, but are not limited to a copolymer poly(ethylene oxide) with poly(L-Lactic acid) or with poly(beta-benzyl-L-aspartate); copolymer with poly(lactide-co-glycolide)-[(propylene oxide)-poly(ethylene oxide)]; polyphosphazene derivatives; poly(ethylene glycol) coated nanospheres; poly(isobutylcyanoacrylate) nanocapsules; poly(gamma-benzyl-L-glutamate)/(poly(ethylene oxide)); chitosan-poly(ethylene oxide) nanoparticles; nanoparticles where the anti-proliferative drug is prepared using o-carboxymethylate chitosan (o-CMC) as wall forming material; solid lipid nanoparticles or nanospheres (SLNs) and any known formulation of nano-particles known to someone skilled in the art.
- 20

- Examples of micro-capsules (or micro-spheres) or formulations therewith include but are not limited to multiporous beads of chitosan; coated alginate microspheres; N-(aminoalkyl) chitosan microspheres; chitosan/calcium alginate beads, poly(adipic anhydride) microspheres; gellan-gum beads; poly(D, L-lactide-co-glycolide) microspheres; alginate-poly-L-lysine microcapsules; crosslinked chitosan microspheres; chitosan/gelatin microspheres; crosslinked chitosan network beads with spacer groups; aliphatic polyesters such as 1,5-diozepan-2-one and D,L-dilactide microspheres; triglyceride lipospheres; polyelectrolyte complexes of sodium alginate chitosan; polypeptide microcapsules; albumin microspheres; and any other micro-capsule (or micro-sphere) formulation known to someone skilled in the art.
- 25
- 30

By using encapsulated inhibitor, the solubility profile of the inhibitor may be changed according to the environment of the formulation. It may thus act as a solubilising agent as mentioned above. An example of its use is when, an inhibitor of the invention is hydrophilic and a slow-release gel is hydrophobic. An encapsulated inhibitor has an advantage that solubilisation does not require chemical coupling of the inhibitor. Thus, an encapsulated inhibitor allows solubility or emulsification in the slow-release agent, so preventing an otherwise unstable formulation.

Furthermore, encapsulation may be used to modulate the release by the slow-release agent (e.g. fine tune or prolong release time). Furthermore, encapsulation may be used to improve intracellular penetration. The advantages of encapsulated formulation may be applied to inhibitor already chemically modified to improve solubility. For example, cholesterol coupled fluoroacetate may be prepared in microcapsules within a slow release gel. The formulation so produced would provide solubility for the inhibitor, slow release modulated by the presence of capsules and active cellular penetration.

Furthermore, encapsulation may be used to modulate release of the inhibitor when a slow-release agent is not present in a composition. For example, when an inhibitor is administered by infusion or injected under high pressure, the composition may comprise one or more TCA cycle inhibitors in the presence of micro- or nano-capsules and/or optionally one or more glycolysis inhibitors in the presence of micro- or nano-capsules. Such composition may reduce the frequency and/or duration of treatment compare with conventional formulations.

One embodiment of the present invention is a composition as mentioned above in which at least one inhibitor is encapsulated in micro- or nano-capsule(s) (or micro- or nano-sphere(s)). According to one aspect of the invention, at least one inhibitor is also pre-coupled to a solubilising agent as mentioned above.

30

Solid wall composition

In cases where the composition is viscous, such as, for example, when a particular slow-release agent is present, it can be difficult to administer into a proliferating mass owing to the force required to move the composition through administering tubing or needle. To make administration easier, the composition may be formed into one or more solid wall entities i.e.

entities having at least solid or semi-solid walls, which entities are small enough to pass through a needle and into the proliferating mass.

According to one aspect of the invention, a solid wall composition is where the composition is enclosed within a solid or semi-solid bioabsorbable membrane to form a contained capsule. Such capsules are of suitable size and shape (small enough) to pass through a needle and into the proliferating mass. Once administered, the capsule dissolves, and the composition is released. Optionally, the composition may also be disposed on the exterior surface of the capsule, and/or impregnated within the membrane of the capsule. The capsule can be spherical, oval, seed-shaped, tubular or any suitable shape for administration using a needle. The capsule wall can be made of any suitable biocompatible and bioabsorbable material such as, for example, gelatine.

According to another aspect of the invention, a solid wall composition is a solid state bioabsorbable structure, impregnated with composition. Such solid state structures are of suitable size and shape (small enough) to pass through a needle and into the proliferating mass. Once administered, the structure dissolves, and the composition is concomitantly released. The structure can be seed-shaped, rod-shaped, tube-shaped or any suitable shape for administration using a needle. The structure can be made of any suitable biocompatible and bioabsorbable material such as, for example, aliphatic polyesters such as homopolymers and copolymers of lactic acid, glycolic acid, lactide, glycolide, para-dioxanone, trimethylene carbonate, epsilon-caprolactone, lactide-capronolactone etc. and blends thereof.

The solid wall composition readily passes through tubing, requiring less force compared with liquid viscous compositions. Furthermore, the precise dose of composition can be administered, and no residual composition is left in the syringe or needle. The solid wall composition can be administered individually, as a series of punctures, for example, one rod per injection. Or, where sufficiently small (nanocapsules), administered in a similar manner to a liquid composition. The maximum width of a solid wall composition is less than the internal diameter of the administering tubing or needle, and can be less than 3 mm, 2 mm, 1 mm, 0.5 mm or a width in the range between any two of the aforementioned widths.

Bone metastases

Another embodiment of the present invention is a composition as described herein further comprising pyrophosphate. Said composition may be used to treat a bone tumour first, by preventing tumour cell proliferation, and in a second step, to stimulate bone reconstruction.

5 The composition may be injected inside a bone metastasis. According to the present pyrophosphate may be any suitable salt of pyrophosphate, including, but not limited to sodium pyrophosphate, potassium pyrophosphate, calcium pyrophosphate. The pyrophosphates may be mixed to the polymer containing the inhibitors. Once the polymer has been degraded and all inhibitors have been absorbed, the presence of pyrophosphates
10 may stimulate new bone formation.

Simultaneous, separate, sequential

According to one aspect of the invention, pyrophosphate may be administered simultaneous, separate or sequentially in respect of the other components of the composition of the
15 invention.

Another aspect of the invention is a composition comprising pyrophosphate and at least one TCA cycle inhibitor and/or at least one glycolysis inhibitor as disclosed herein, for simultaneous, separate or sequential administration to a subject.

20

One aspect of the invention is a method for treating cellular proliferation comprising administering to an individual an effective amount of pyrophosphate and at least one TCA cycle inhibitor of the invention and/or at least one glycolysis inhibitor, simultaneously, separately or sequentially.

25

For example, a method for treating cellular proliferation in a bone (for instance, a metastasis) comprises administering to an individual an effective amount of pyrophosphate and at least one TCA cycle inhibitor of the invention and/or at least one glycolysis inhibitor, simultaneously, separately or sequentially.

30

By simultaneous administration means the pyrophosphate and TCA cycle inhibitor and/or glycolysis inhibitor are administered to a subject at the same time. For example, as a mixture or a composition comprising said components. An example is as a solution comprising the pyrophosphate and TCA cycle inhibitor and glycolysis inhibitor. Another example is as a slow

release polymer comprising the pyrophosphate and TCA cycle inhibitor and glycolysis inhibitor.

By separate administration means the pyrophosphate and TCA cycle inhibitor and/or glycolysis inhibitor are administered to a subject at the same time or substantially the same time. The components may be present in a kit as separate, unmixed preparations. For example, the pyrophosphate, TCA cycle inhibitor and glycolysis inhibitor may be present in the kit as individual vials. The inhibitors may be administered to the subject by separate injections at the same time, or injection directly following the other.

10

By sequential administration means the pyrophosphate and TCA cycle inhibitor and/or glycolysis inhibitor are administered to a subject sequentially. The pyrophosphate and TCA cycle inhibitor and/or glycolysis inhibitor may be present in a kit as separate, unmixed preparations. There is a time interval between doses. For example, one component might be administered up to 336, 312, 288, 264, 240, 216, 192, 168, 144, 120, 96, 72, 48, 24, 20, 16, 12, 8, 4, 2, 1, or 0.5 hours after the other component.

15

In sequential administration, one component may be administered once, or any number of times and in various doses before and/or after administration of another component.

20

Sequential administration may be combined with simultaneous or sequential administration.

Combined radiotherapy or chemotherapy treatment

Another aspect of the invention, is a method of treating proliferating cells comprising delivering to proliferating cells a composition according to the invention, and radiotherapy and/or chemotherapy. The use of the composition can lead to effective treatment using a fraction of the normal radiotherapy or chemotherapy therapeutic dose.

25

According to this aspect of the invention, proliferating cells are treated by administering a composition locally, preferably into the proliferating cell mass, as mentioned above.

30

According to this aspect of the invention, a tumour is totally or partially resected and an implant is placed inside the resection cavity as mentioned above. The site of the proliferation is treated with radiotherapy applied either from an exterior source, or by the introduction of radioactive sources inside the tumour (brachytherapy). The radioactive source may also be a radio-isotope-halogen (RIH) inhibitor as described above. The combination of locally

administered composition and radiotherapy treatments may lead to a rapid and effective shrinking or death of the proliferation.

According to this aspect of the invention, proliferating cells are treated by administering a
5 composition locally, preferably into the proliferating cell mass, inside the tumour, as
mentioned above, and in addition, the site of the proliferation is treated with intravenous
chemotherapy (for instance paclitaxel, cisplatin, vinorelbine, etc). The combination of
locally administered composition and radiotherapy and/or chemotherapy treatments may
lead to a rapid and effective shrinking or death of the tumour. It is expected that
10 chemotherapy and/or radiotherapy will be much more efficient after the local application of
the inhibitors inside the proliferating process. It is foreseen that accumulated doses of
radiotherapy and/or chemotherapy could be decreased from 10 to 50 %.

One embodiment of the present invention is a method for treating cellular proliferation
15 comprising administering a composition locally, preferably into the proliferating cell mass,
as described herein in combination with radiotherapy.

One embodiment of the present invention is a method for treating cellular proliferation
comprising administering a composition locally, preferably into the proliferating cell mass,
20 as described herein in combination with chemotherapy.

Another embodiment of the present invention is a method for reducing the dose of
radiotherapy treatment of a tumour, comprising administering a composition locally,
preferably into the proliferating cell mass, as mentioned above prior to radiotherapy.
25

Another embodiment of the present invention is a method for reducing the dose of
chemotherapy treatment of a tumour, comprising administering a composition locally,
preferably into the proliferating cell mass as mentioned above prior to chemotherapy.

30 Where radiotherapy and chemotherapy are administered, the composition of the present
invention may be used to reduce both radiotherapy and chemotherapy doses. A typical
chemotherapy and/or radiotherapy dose may be about 20%, 30%, 40%, 50%, 60%, 70%,
80%, 90% less than the dose normally applied to a tumour, in view of the size, location and
other factors. It may be a value in the range between any two of the aforementioned values.
35 Preferable, the dose is between 20 and 70% less than the normal dose.

Another embodiment of the present invention is a method for sensitising a proliferating cell mass (e.g. tumour) to radiotherapy, comprising administering a composition locally, preferably into the proliferating cell mass as mentioned above prior to radiotherapy.

5

Another embodiment of the present invention is a method for sensitising a proliferating cell mass (e.g. tumour) to chemotherapy, comprising administering a composition locally, preferably into the proliferating cell mass as mentioned above prior to chemotherapy.

- 10 According to one aspect of the invention, the composition is applied to a subject at least 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 1 day, 2 days, 3 days, 4 days, 6 days, 8 days, 10 days, 12 days, 14 days, 3 weeks or 4 weeks before the start of radio- and/or chemotherapy, or for a period between any two of the aforementioned periods. Preferably, the composition is in place for between 12 hours to 4 weeks before
15 commencement of radio- and/or chemotherapy.

Dose

The quantity and concentration of composition for injection and the frequency of administration can be calculated using known techniques by the skilled person.

20

- Typically, 1 to 3-4 grams of the slow release substance may be administered to a 30-50 grams tumor. The injections may be made in several places inside or proximal to the tumor, in order to warrant an homogeneous distribution of the substances. For instance a 30 grams cervical lymphnode from a tonsillar tumor would receive under ultrasonic control 3 injections
25 of 1 gram of composition in 3 different places of the lymphnode. Each gram of the composition would contain 1 milligrams of fluoroacetate and 100 milligrams of oxamate, for instance. If taking a tri-block copolymer with a release period of 1 month, the patient is re-examined after 1 month. If the lymphnode did not disappear, but has shrunk to half of its initial size, 1 to 2 injections of 1 gram of the polymer formulation may be repeated 1 month
30 after the first injection, and again 1 month later if necessary to kill all tumor cells.

- The active substance may be deposited as a narrow (e.g. 1 to 1.45 mm diameter) cylinder of paste extruded from a needle or tube into the proliferating mass. The polymer will slowly release the product. If several such cylinders are deposited inside a lesion, it ensures a
35 better homogeneity for the therapy. A person skilled in the art will take into account the

release rate of the polymer (for instance 5 days or 6 weeks), in order to clinically observe the effects of the treatment. After all the drug has been released, the effect should be considered as being maximal. A repeated therapy will be decided if necessary.

- 5 Some cancers are present as huge masses, for instance 5 kgs or 18 kgs. In these cases, the local therapy could be performed as a intra-arterial infusion first, in order to decrease the tumoral volume. This infusion could be combined with standard chemotherapy as well (paclitaxel, cisplatinum, bleomycin, vinorelbin, etc.). When the volume has decreased, after several weeks, the tumor may be implanted with a slow-release polymer containing an active
10 substance in a second step.

- According to one aspect of the invention, a composition comprises TCA inhibitor such that the inhibitor concentration delivered to a subject is greater than or equal to 1, 10, 20, 40, 60, 80, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or mg inhibitor / kg of tumour or
15 of treated mass, or a concentration in the range between any two of the aforementioned values. Preferably the dose is between 1 and 200 mg/kg of tumour or of treated mass.

- According to one aspect of the invention, a composition comprises glycolysis inhibitor such that the inhibitor concentration delivered to a subject is greater than or equal to 20, 40, 60, 80, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000 or mg inhibitor / kg
20 of tumour or of treated mass, or a concentration in the range between any two of the aforementioned values. Preferably the dose is between 0.02 and 2 g/kg of tumour or of treated mass.

25 *Imaging agents*

- Another embodiment of the present invention is a composition further comprising one or more imaging agents which allow the composition to be viewed using an *in vivo* imaging device. According to one aspect of the invention, a composition further comprises a magnetic-resonance-visible agent, such as, for example, poly(ortho)ester, metallic powder
30 (e.g. tantalum powder), or any other MR visible agent (magnesium alloy powder). According to one aspect of the invention, a composition further comprises a radio-opaque agent, such as, for example, biocompatible metal powder (e.g. tantalum, iridium powder), or any other agent appear opaque to X-rays. According to another aspect of the invention, a composition further comprises micro-bubbles in order to render the composition ultrasound visible.

The addition of imaging agents allows a physician to accurately administer the composition with the assistance of an *in vivo* imaging device, and also to follow the distribution in the proliferating cells thereafter.

5 *Combinations*

A composition comprising one or more of the aforementioned components is within the scope of the present invention. For example, a composition may comprise one or more TCA cycle inhibitors optionally coupled to solubilising agent, one or more glycolysis inhibitors optionally coupled to solubilising agent, one or more slow-release agents, one or more
10 micro- or nano-particles, one or more one or more sensitising agents, and/or one or more imaging agents, and other components known to the skilled person for suitable formulation of the composition. As mentioned above, combinations allow blocking many pathways simultaneously, avoiding shuttles compromising the effect of isolated inhibitors.

15 The inventors have also found that inhibition of glycolysis and TCA cycle is effective against proliferating cells; proliferating cells do not obtain energy *via* other pathways and recover. *In vitro* data shows separate administered inhibitors inhibit growth of cancer cells growth, *in vivo*, given by intravenous route they are not efficacious to treatment tumours such as prostate cancer. Inhibitors of glycolysis and TCA cycle can be toxic to a subject and have
20 been largely overlooked for effective treatment of conditions such as cancer. The doses needed for efficacy *via* the systemic route, such inhibitors would be very high. By locally delivering a combination of inhibitors into proliferating cells in a slow release formulation, the delivery period is prolonged *i.e.* the inhibitors are not cleared by the liver, and the dose received by the tumour is effectively higher than using systemic delivery. Furthermore, the
25 lethal dose can be greatly exceeded. Because of the high metabolic rate of proliferating cells, certain inhibitors are rapidly and selectively taken up the proliferating cells and not by the healthy cells. Thus, a composition comprising the present inhibitors in a slow release formulation increases the effective dose to the tumour and permits greater than lethal dosing. Furthermore, the combination of inhibitors leads to a surprisingly effective inhibition of
30 proliferation.

Furthermore, the composition may be used in combination with radiotherapy, where a lower dose of inhibitor may be used.

It is possible to select which pathway of glycolysis or TCA is better to be shut down by performing a 2-FDG and a ^{11}C -acetate positron emission tomography examination, and evaluating the activity of glycolysis and TCA cycles in the said tumour, allowing to choose for each individual tumour which compound should be favoured for the inhibition.

5

The combination of ATP inhibitors is very important for the inventors. The inventor,s own clinical experience (MR spectroscopy and PET-CT examinations) as well as data from the literature confirm the great variety of substrates taken up by tumours. A review of the literature data shows for instance that the affinity for 18-FDG intake varies from 3 to 100 %, depending on evaluated tumours and affected organs (e.g. http://www.petscaninfo.com/zportal/portals/physis/clinical/jnmpetlit/index_html/JNM_OncoApps/JNM_Table8/article_elements_view). The inventors have also observed that a beneficial therapeutic approach is to treat several ATP synthesis pathways simultaneously.

15 *Kit*

A kit according to the invention comprises at least one composition of the present invention.

It is an aspect of the invention that a composition is provided in a container. For example, a vial, a sachet, a screw-cap bottle, a syringe, a non-resealable vessel, a resealable vessel. Such containers are any that are suitable for containing a composition. Some active products, for example, are sensitive to light and heat and should be preserved in dark and cold.

A kit may provide a range of vials containing different compositions with different inhibitors, different combinations of inhibitors, different combinations of slow-release polymers. A kit may comprise a means for administering the composition (e.g. one or more syringes). A kit may facilitate the sequential application of more than one type of composition. A kit may contain instructions for use.

30 **EXAMPLES**

The invention is illustrated by the following non-limiting examples. They illustrate the effectiveness of a selection of TCA cycle and glycolysis inhibitors described above. The inhibitory properties of the other inhibitors not mentioned in the examples are known, and the skilled person may readily substitute the exemplified inhibitors with equivalent pathway inhibitors such as listed above.

Example 1

A patient has a prostate carcinoma, a PSA level at 10 ng/ml, prostate biopsies showing a
5 gleason score of 7 and lesions present in both lobes. A fluor-choline PET-CT does not show
any pathologic lymphnodes in the pelvis. This patient is a candidate for a local therapy such
as surgical resection or external or interstitial radiation therapy. Another alternative is to
implant the peripheral area of the prostate under ultrasound, MR or CT control with a slow
10 release formulation of fluoroacetate, oxamate or 2-FDG (fluorodeoxyglucose), or iodoacetate
or a combination of products. The patient undergoes loco-regional anesthesia and is placed
in gynecological position. The implantation may be performed under an open MR machine or
using ultrasonic control. A mixture of poly(ortho)ester and fluoroacetate is injected inside the
prostate, in the prostatic peripheral area, several mm inside the prostate capsule (5 to 10
15 mm). 1 to 4 mg of fluoroacetate in total in slow release formulation are injected as 8
peripheral injections. Simultaneously, 6 other injections are performed with 200 mg of 2-
fluorodeoxyglucose or 300 mg oxamate (or 100 mg of iodoacetate) between the injection
areas of fluoroacetate. As the poly(ortho)ester (POE) is perfectly visualized under MR, one
can follow deposition areas, and during following days, the degradation of the POE. The
20 injected POE degrades over 10 to 30 days, depending on the local biological conditions. The
fluoroacetate and/or oxamate or 2-FDG, or iodoacetate are actively absorbed by the tumor
cells. In the following months the therapeutic effect is observed by measuring the PSA blood
levels. In case of tumor persistence, a second or a third therapeutic session is realised. The
exact tumor area is determined by MR spectroscopy or PET-CT, and this area is implanted
exclusively and repeatedly. It is supposed that the injected drug follow the lymphatic
25 pathways and treats microscopic foci in drainage lymphnodes as well.

Example 2

A 88 y old patient presents with a biopsy-proven cervix uteri tumor, 3 cm in diameter. No
lymphnodes are seen on the MR or PET-CT examinations. The 11-Cacetate PET-CT
30 examination shows high tumoural uptake with a SUV (standard uptake value) of 5. There are
several standard therapeutic alternatives: (a) the patient is operated on, and the lymphnodes
are removed (b) the patient receives radiation therapy simultaneously with a cisplatin based
chemotherapy. Finally the patient refuses the aforementioned treatments and she benefits
from one to several injections of fluoroacetate (1-4 mg in total) and FDG (2-300mg in total) or
35 oxamate (3-400 mg in total) in a slow release formulation. Another possibility is to place a

- tube with holes inside the cervix, and to inject same doses of fluoroacetate and oxamate under high pressure (2 to 5 000 Atm) from inside the cervical canal, perpendicularly to cervical canal axis, towards the tumor through a very thin catheter with micrometric holes (200 microns catheter, and 50 microns holes). After several such injections, the tumor
- 5 shrinks and disappears. The patient is carefully observed, for loco-regional recurrence and for a recurrence in the lymphnodes.

Example 3

- A patient presents with a 4 cm diameter pulmonary mass located in the left inferior lobe.
- 10 There are bilateral lymphnodes in the mediastinal area and the patient is not considered for a surgical intervention. In order to avoid irradiating the lung tissue that surrounds the primary tumor, an injection of 1-4 mg of slow release fluoroacetate and 2 – 300 mg of FDG is performed inside the tumoral mass, under CT guidance. This drug is delivered over the next
- 15 10 days. In parallel, a standard cisplatin based chemoradiotherapy is started to treat the primitive tumour and the mediastinal lesions. Some of the mediastinal invaded lymphnodes may be implanted with the slow release composition as well, if easily reachable under CT guided puncture. The radiation dose needed to sterilize the injected lesions is possibly reduced from 70-75 to 40-60 Gy. The patient is carefully observed by regular Ct scans.

20 Example 4

- A 30 y old patient presents with a cheloid scar. The patient is operated, the cheloid scar is resected, and a catheter is left at the bottom of the scar for several days. After 1-2 weeks, when the sealing process of the scar is underway, a slow release polymer (lactide-capronolactone), shaped as a thin rod, is deposited at the bottom of the resected area using
- 25 the catheter that was left in place, preventing the cheloid scar formation, which usually initiates at the bottom of the scar. The amount of fluoroacetate delivered at the bottom of the scar is in the range of 1 to 100 micrograms per cm of scar length. Fluoroacetate may be combined with 2-FDG (1-10 mg per cm) or oxamate (5 to 20 mg per cm) for instance.

30 Example 5

- A 80 years old patient has been operated for a 1cm breast cancer (quadrantectomy). The sentinel node technique did not show any invasion. In place of undergoing external beam radiation of the breast, the patient receives an injection of slow release fluoroacetate (1-4 mg) and 2-FDG (300 mg), 3 weeks after the surgical excision, inside the surgical scar, under

US, CT or MR guidance. This treatment will allow external beam radiotherapy to be avoided, sharply reducing the recurrence rate.

Example 6

- 5 A 33 year old woman presents with a 6 cm diameter myoma in the uterine wall, a benign tumor, that grows approximately 2 cm in diameter per year. The standard therapy is the surgical resection of the myoma mass. In the present case, 3-5 mg of fluoroacetate and 400 mg 2-FDG prepared in a slow release formulation are injected twice, at 1 month interval, into the myoma mass. This leads to a sharp decrease of the myoma size after several weeks and
10 avoids the need for a surgical intervention.

Example 7

- A 44 year old patient presents with a 2 cm long oesophageal tumor. The echoendoscopic examination shows a 8 mm thick circumferential tumor. A flexible injector is introduced inside
15 the biopsy channel of the endoscope and 4 punctures are performed all around the esophageal circumference, through angulated punctures, inside the tumoral thickening. 1 to 5 mg of fluoroacetate and 400 mg oxamate in a slow release formulation are delivered. The patient is then submitted to chemotherapy and radiation. Radiation is delivered at a reduced therapeutic dose of 50 Gy instead of 70 Gy and the tumor is totally eradicated.

20

Example 8

- A 45 y old woman complains from uterine bleeding. An MRI examination shows 3 benign myomas located in the uterine wall, 5, 3 and 2.5 cm in diameter. The patient undergoes locoregional anesthesia, and under ultrasonic or MRI control all three lesions are punctured
25 and injected with a POE polymer, 1 cc each, each containing oxamate, 200 mg per syringe and fluoroacetate embedded in solid-lipid-nanoparticles (SLNs), with 2-3 mg of fluoroacetate per g of poly(ortho)ester (POE). The myomas reduce in size and the patient is followed-up.

Example 9

- 30 A 40 year old patient presents with a malignant tumor in the right tonsillar area, 2 cm in diameter, with a lymphnode of 3 cm of diameter in the right cervical area. The patient refuses surgery and radio-chemotherapy. 1 g of poly(ortho)ester (POE) is injected inside each lesion. The formulation contains 300 mg of 2-FDG and 3 mg of fluoroacetate attached to cholesterol, mixed to the POE, per gram of POE. The injection are repeated 3 times at 1
35 month interval. The lesions disappear and the patient is regularly followed up for recurrence.

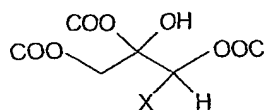
Example 10

A 40 y old patient presents with a basal carcinoma of the skin, 1 cm in diameter, located in the right side of his thorax. A hydrogel foil, 3 mm thick, 2.5 cm in diameter containing 10 mg per square cm of oxamate and 0.1 mg per square cm of fluoroacetate is deposited on the lesion and fixed with a translucent adhesive bandage. The foil is changed every day during 2 weeks. At 6 weeks the patient is examined showing only a scar at the place where the lesion was present. The patient is followed up.

CLAIMS

1. Use of a composition comprising one or more inhibitors of the citric acid, TCA, cycle and/or one or more inhibitors of glycolysis for the manufacture of a medicament for the treatment of cellular proliferation, wherein said composition is administered into a mass of the proliferating cells.
2. Use according to claim 1, wherein said TCA cycle and glycolysis inhibitors are administered separately, simultaneously or sequentially .
3. Use according to claims 1 or 2, wherein said TCA cycle inhibitor is an inhibitor of one or more of pyruvate dehydrogenase, citrate synthase, aconitase, isocitrate lyase, alpha-ketoglutarate dehydrogenase complex, succinyl CoA synthetase, succinate dehydrogenase, fumarase, malate synthase, glutaminase and pyruvate dehydrogenase complex.
4. Use according to any of claims 1 to 3, wherein said TCA cycle inhibitor is any of arsenite, hypoglycin A, methylenecyclopropylacetic acid, alloxan, PNU, p-benzoquinone, fluoroacetate, halogenated acetates (iodo-, bromo-, chloro-acetate), halogenated acetyl-CoA (fluoroacetyl-CoA, bromoacetyl-CoA, chloroacetyl-CoA, iodoacetyl-CoA), halogenated crotonate (fluoro-, iodo-, bromo-, chloro-crotonate), halogenated ketone bodies, (chloro-, fluoro-, bromo-, iodoaceto-acetate, fluoro-, chloro-, bromo-, iodo-butyrates, fluoro-, chloro-, bromo-, iodo-acetone), halogenated oleate (iodo, bromo, chloro, fluoro-oleate), halogenated citrate, halogenated citrate 2R, 3R isomer (fluoro-, bromo-, chloro-, iodo-citrate), dichlorovinyl-cysteine, halogenated aminoacids, malonate, pentachlorobutadienyl-cysteine, 2-bromohydroquinone, 3-nitropropionic acid, cis-crotonalide fungicides, glu-hydroxyoxamate, p-chloromercuriphenylsulphonic acid, L-glutamate gamma-hydroxamate, p-chloromercuriphenylsulphonic acid, acivicin (alpha-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid), halogenated glutamine (fluoro, iodo, chloro, bromo-glutamine), or halogenated glutamate (fluoro, iodo, chloro, bromo-glutamate), a stereoisomer, tautomer, racemate, prodrugs, metabolite thereof, or a pharmaceutically acceptable salt, base, ester or solvate thereof.
5. Use according to any of claims 1 to 3, wherein said TCA cycle inhibitor is a compound of formula (I) or a stereoisomer, tautomer, racemate, prodrug, metabolite thereof, or a pharmaceutically acceptable salt, base, ester or solvate thereof:

47



(I)

where X is halide, a sulfonate, a carboxylate, an alkoxide, an amine oxide or OH.

5

6. Use according to claim 5, wherein formula (I):

- a halide is selected from the group consisting of: fluoride, bromide, chloride, and iodide,

10 - a sulfonate may be selected from the group consisting of: triflate, mesylate and tosylate,

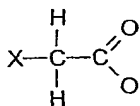
- a carboxylate may be selected from the group consisting of: methoxylate and ethoxylate,

- an alkoxide may be selected from the group consisting of: methoxide and ethoxide,

- an amine oxide is dimethylamine oxide, and

15 - where the stereochemistry is 2R, 3R,

7. Use according to claims 1 to 3, wherein said TCA cycle inhibitor is a compound of formula (II) or a stereoisomer, tautomer, racemate, prodrug, metabolite thereof, or a pharmaceutically acceptable salt, base, ester or solvate thereof:



20

(II)

where X is a halide, a sulfonate, a carboxylate, an alkoxide, an amine oxide, or an OH.

8. Use according to claim 7, where in formula (II):

25 - the halide is selected from the group consisting of: fluoride, bromide, chloride, and iodide,

- the sulfonate is selected from the group consisting of: triflate, mesylate and tosylate,

30 - the carboxylate is selected from the group consisting of: methoxylate and ethoxylate,

- the alkoxide is selected from the group consisting of: methoxide and ethoxide, and
- the amine oxide is dimethylamine oxide.

9. Use according to any of claims 1 to 3, wherein an inhibitor of the TCA cycle is any of
5 fluoroacetate, fluorocitrate, arsenite, acetoacetate, and betahydroxy butyrate.

10. A composition according to any of claims 1 to 9, wherein said TCA cycle inhibitor, when comprising a halogen atom, comprises a halogen radioisotope in place of a stable halogen atom.

10

11. Use according to any of claims 1 to 10, wherein said inhibitor of glycolysis inhibits at least one enzyme from the group consisting of hexokinase, glucokinase, phosphoglucose isomerase, phosphofructokinase, aldolase, triose phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglyceromutase, enolase,
15 pyruvate kinase, and lactate dehydrogenase.

12. Use according to any of claims 1 to 11, wherein said inhibitor of glycolysis is a hexose sugar modified by removal of the hydroxyl group or by the substitution of the hydroxyl group with halogen atom or thiol at:

20

- C6,
- C1 or C2 or C5,
- C3 and/or C4, and/or
- C2 or C3.

25

13. Use according to any of claims 1 to 11, wherein said inhibitor of glycolysis is any of 6-deoxy-6-fluoro-D-glucose, 6-deoxy-6-bromo-D-glucose, 6-deoxy-6-chloro-D-glucose, 6-O-methyl-D-glucose, 6-thio-D-glucose, 6-deoxy-D-glucose, C-6 modified or blocked derivatives of other hexose ring pyranoses, mannopyranoses, galactopyranoses, 6-deoxy-6-fluoro-D-glucose, 6-deoxy-6-bromo-D-mannose, 6-deoxy-6-chloro-D-mannose, 6-deoxy-6-fluoro-D-galactose, 6-deoxy-6-chloro-D-galactose, 6-deoxy-6-iodo-D-galactose, 6-deoxy-6-bromo-D-galactose, halogenated C-6 sugars gluconolactones, glucuronic acid, glucopyranoside, and their phosphate derivatives, glucuronides with halogenated glycosides at the C-1 position, C-2 substituted D-hexoses, 2-deoxy-2-halogeno-D-hexoses, 2-deoxy-2-fluoro-D-glucose, 2-chloro-2-deoxy-D-glucose, 2-bromo-D-glucose, 2-iodo-D-glucose, 2-deoxy-2,2-difluoro-D-arabino-hexose, 2-deoxy-2-fluoro-D-mannose, 2-deoxy-D-arabino-hexose, 2-Deoxy-2-fluoro-
35

- D-galactose, 1,6-anhydro-2-deoxy-2-fluoro-beta-D-glucopyranose, 1-6-anhydrosugar, 2-amino-2-deoxy-D-glucose, glucose amine, 2-amino-2-deoxy D-galactose, galactosamine, 2-amino-2-deoxy-D-mannose, mannosamine, 2-deoxy-2-fluoro-D-mannose, 2-deoxy-2-fluoro-D-galactose, 2-deoxy-D-arabino-hexose, 2-deoxy-2,2-difluoro-D-arabino-hexose, 2-deoxy-2-fluoro-D-glucose 1-Phosphate, 2-deoxy-2-fluoro-D-glucose 6-P, 2-deoxy-2-fluoro-D-glucose 1,6 biphosphate, 2-deoxy-2-fluoro-D-mannose 1-P, 2-deoxy-2-fluoro-D-mannose 6-P, 2-deoxy-2-fluoro-D-mannose 1,6-biphosphate, nucleotide diphosphate, uridine di-P, 1-2 deoxy-2-fluoro-D-glucose, C-2-halogen substituted, and NH₃ substituted derivatives of D-Glucose 6-phosphate, 2-deoxy-2-fluoro-2-D-glucose-6-phosphate, 2-chloro-2-deoxy-D-glucose-6-phosphate, 2-deoxy-D-arabino-hexose-6-phosphate, D-glucosamine-6-phosphate, 2-deoxy-2-fluoro-2-D-manose-6-P, and any known derivatives, C-2 halogenated derivatives of hexose ring pyranoses, mannopyranoses, galactopyranoses, C-2-deoxy-2- fluoropyranoses, and any derivative, C-2 halogenated sugars derivatives, C-2 fluoro-, bromo-, chloro-, or iodo-sugars derivatives, fluoro, bromo, chloro, or iodo C-2 sugars derivatives, gluconolactones, glucuronic acid, glucopyranoside, and their phosphate derivatives, sugars modified at C-1 or C-5 by replacement of hydroxyl by fluorine or deoxygenation or replacement by a sulfur group, glucosyl fluoride, 1-deoxy-D-glucose, 5-thio-D-glucose, 3-deoxy or 3-fluoro-D-glucose or 4-deoxy or 4-fluoro-D-glucose, 2-fluoro- or 2-iodo-, or 2-thio-, or 2-methoxy- or 3-fluoro-, or 3, 3 difluoro-, 3-iodo-, or 3-carboxylo-, or 3-thio-glyceraldehydes or glycerates, 3-fluoro-2-phosphoglycerate, phosphothioesters or other phosphor modified analogs, mannoheptulose mannoheptose, glucoheptose, N-acetylglucosamine, 6-aminonicotinamide acidosis-inducing agents, 2-deoxy-2-fluoro-D-glucose, citrate and halogenated derivatives of citrate, fructose 2,6-bisphosphate, bromoacetyethanolamine phosphate analogues, N-(2-methoxyethyl)-bromoacetamide, N-(2-ethoxyethyl)-bromoacetamide, N-(3-methoxypropyl)-bromoacetamide), iodoacetate, pentalenolactone, arsenic, 1,1-difluoro-3-phosphate-glycerol, oxamate, 2-fluoro-propionic acid or it salts, 2,2-difluoro-propionic acid, 3-halopropionic acid, or 2-thiomethylacetic acid, a stereoisomer, tautomer, racemate, prodrug, metabolite thereof, or a pharmaceutically acceptable salt, base, ester or solvate thereof.
14. A composition according to any of claims 1 to 11, wherein an inhibitor of glycolysis is any of 2FDG, oxamate or iodoacetate or a stereoisomer, tautomer, racemate, prodrug, metabolite thereof, or a pharmaceutically acceptable salt, base, ester or solvate thereof.

15. A composition according to any of claims 1 to 14, wherein said glycolysis inhibitor, when comprising a halogen atom, comprises a halogen radioisotope in place of a stable halogen atom.
- 5 16. Use according to any of claims 1 to 15, wherein said composition further comprises pyrophosphate.
17. Use according to claim 16, wherein said pyrophosphate is one or more of sodium pyrophosphate, potassium pyrophosphate, calcium pyrophosphate.
- 10 18. Use according to claims 16 or 17, wherein said pyrophosphate is administered simultaneous, separate or sequentially in respect of the other components of the composition.
- 15 19. Use according to any of claims 1 to 18, wherein said composition further comprises one or more imaging agents
20. Use according to any of claim 19, wherein said imaging agent is any of poly(ortho)ester, metallic powder, magnesium alloy powder, tantalum powder, biocompatible metal powder, iridium powder, or micro-bubbles.
- 20 21. Use according to any of claims 1 to 20, wherein said composition further comprises one or more slow releasing agents.
- 25 22. Use according to claim 21, wherein said slow release agents is a polymer that is any of magnesium alloy, poly(glycolic) acid, poly(lactic acid) or in general glycolic- and lactic acid based polymers, copolymers, poly caprolactones and in general, poly hydroxyl alkanoate,s poly(hydroxy alcanoic acids), Poly (ethylene glycol), poly vinyl alcohol, poly (orthoesters), poly (anhydrides), poly (carbonates), poly amides, poly imides, poly imines, poly (imino carbonates), poly (ethylene imines), polydioxanes, poly oxyethylene (poly ethylene oxide), poly (phosphazenes), poly sulphones, lipids, poly acrylic acids, poly methylmethacrylate, poly acryl amides, poly acrylo nitriles (Poly cyano acrylates), poly HEMA, poly urethanes, poly olefins, poly styrene, poly terephthalates, poly ethylenes, poly propylenes, poly ether ketones, poly vinylchlorides, poly fluorides, silicones, poly silicates (bioactive glass),
- 30 35 siloxanes (Poly dimethyl siloxanes), hydroxyapatites, lactide-capronolactone, poly

aminoacids (natural and non natural), poly β -aminoesters, albumines, alginates, cellulose / cellulose acetates, chitin / chitosan, collagene, fibrine / fibrinogen, gelatine, lignine, proteine based polymers, Poly (lysine), poly (glutamate), poly (malonates), poly (hyaluronic acids), Poly nucleic acids, poly saccharides, poly (hydroxyalkanoates), poly isoprenoids, starch
5 based polymers, copolymers thereof, linear, branched, hyperbranched, dendrimers, crosslinked, functionalised derivatives thereof, hydrogels based on activated polyethyleneglycols combined with alkaline hydrolyzed animal or vegetal proteins.

23. Use according to any of claims 1 to 22, wherein at least one of said inhibitors is coupled
10 to solubilising agent.

24. Use according to claim 23, wherein said solubilising agent is cholesterol or derivative thereof.

15 25. Use according to claim 24, wherein said cholesterol derivatives are any of cholesteryl-3-betahydroxybutyrate, cholesteryl-halogenated butyrate, cholesteryl-halogenated acetate, cholesteryl-halogenated aceto-acetate, cholesteryl-halogenated acetamide, cholesteryl-halogenated crotonate, cholesteryl-halogenated acetone, cholesteryl-halogenated citrate, or cholesteryl-halogenated oleate

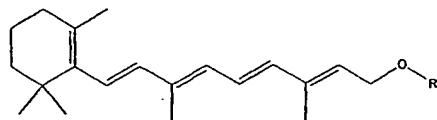
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26. Use according to claim 23, wherein solubilising agent is vitamin A or derivative thereof.

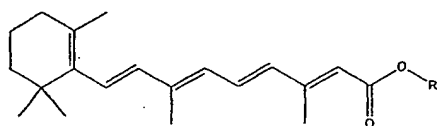
27. Use according to claim 26, wherein derivative of vitamin A is formula (IV) or (V):

25

30



(IV)



(V)

wherein R is selected from the group consisting of betahydroxybutyrate, halogenated butyrate, halogenated acetate, halogenated aceto-acetate, halogenated acetamide, halogenated crotonate, halogenated acetone, halogenated citrate, and halogenated oleate.

28. Use according to any of claims 1 to 27, wherein at least one of said inhibitors is present in micro-capsule and/or nano-capsule.

29. Use according to claim 28, wherein nano-capsule is any of copolymer poly(ethylene oxide) with poly(L-Lactic acid) or with poly(beta-benzyl-L-aspartate), copolymer with poly(lactide-co-glycolide)-[(propylene oxide)-poly(ethylene oxide)], polyphosphazene derivatives, poly(ethylene glycol) coated nanospheres, poly(isobutylcyanoacrylate) nanocapsules, poly(gamma-benzyl-L-glutamate)/(poly(ethylene oxide)), chitosan-poly(ethylene oxide) nanoparticles, nanoparticles where said inhibitor is prepared using o-carboxymethylate chitosan as wall forming material, or solid lipid nanospheres (SLN).

30. Use according to claim 28, wherein micro-capsule is any of multiporous beads of chitosan, coated alginate microspheres, N-(aminoalkyl) chitosan microspheres, chitosan/calcium alginate beads, poly(adipic anhydride) microspheres, gellan-gum beads, poly(D, L-lactide-co-glycolide) microspheres, alginate-poly-L-lysine microcapsules, crosslinked chitosan microspheres, chitosan/gelatin microspheres, crosslinked chitosan network beads with spacer groups, 1,5-dioxepan-2-one microspheres, D,L-dilactide microspheres, triglyceride lipospheres, polyelectrolyte complexes of sodium alginate chitosan, polypeptide microcapsules, or albumin microspheres.

31. Use according to any of claims 1 to 30, wherein said composition is part of a solid wall composition.
- 5 32. Use according to claim 31, where said solid wall composition is a capsule of suitable size and shape for administration using a needle, said capsule filled with composition.
33. Use according to claim 32, wherein a wall of said capsule comprises gelatin.
- 10 34. Use according to claim 31, wherein said solid wall composition is a solid state bioabsorbable structure of suitable size and shape for administration using a needle, said structure impregnated with composition.
35. Use according to claim 34, wherein said solid state bioabsorbable structure is seed-
15 shaped, rod-shaped, or tube-shaped.
36. Use according to any of claims 1 to 35, further combined with radiotherapy.
37. Use according to any of claim 1 to 35, further combined with chemotherapy.
- 20 38. Use according to any of claims 1 to 37, wherein said composition is administered by injection into a mass of proliferating the cells.
39. Use according to any of claims 1 to 37, wherein said composition is administered by
25 infusion into a mass of the proliferating cells.
40. Use according to any of claims 1 to 37, wherein said composition is administered by high-pressure injection into a mass of the proliferating cells.
- 30 41. Use according to any of claims 1 to 40, wherein said composition is administered in the resection cavity or scar of a mass of the proliferating cells.
42. Kit comprising a composition comprising one or more inhibitors of the TCA cycle and/or one or more inhibitors of glycolysis.

43. A kit according to claim 32, wherein said composition is a composition as defined in any of claims 1 to 35.

44. A kit according to any of claims 42 or 43, further comprising a syringe.

5

45. A use of a composition as defined in any of claims 1 to 35, wherein a inhibitor of TCA cycle and said proliferating cells are dysplasia of the cervix uteri.

46. A hydrogel comprising a) composition as defined in any of claims 1 to 30, and

10 b) an activated polyethyleneglycol (PEG) combined with any of alkaline hydrolyzed soya solutions, animal or vegetal proteins, bovine serum albumin, soya globulin, casein, pea albumin, starch albumine, or ovalbumin.

15 47. A hydrogel according to claim 46 wherein a TCA inhibitor of the composition is present at a concentration of less than or equal to 0.1 mg per square cm of hydrogel and/or a glycolysis inhibitor of the composition is present at a concentration of less than or equal to 10 mg per square cm of hydrogel.

20 48. A use of a hydrogel according to claim 46 or 47 for treatment of superficial cell proliferation, such as basal carcinoma or a squamous cell carcinoma by application of the hydrogel to the surface of said proliferations.

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